## Acute Leukaemia

**P400**

**Unmanipulated haploidentical bone marrow transplantation for high-risk haematological malignancies using myeloablative conditioning and high-dose post transplantation cyclophosphamide**

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Background: Promising results have been reported with post-transplant HD CY - as GVHD prophylaxis - after non-myeloablative haploidentical BMT (Luznik, BBMT 2008). We evaluated the safety and efficacy of HD-CY post BMT, after myeloablative conditioning and unmanipulated haploidentical BMT, in patients with advanced or refractory hematologic malignancies.
End point of the study: In this study we assessed hematologic engraftment, non relapse mortality (NRM), together with acute GVHD and infections, and relapse related death (RRD).
Patients: We report 40 patients (median age 42, range 16-66); the diagnosis was AML (n=19), ALL (n=10), CML blast crisis (n=2), LNH (n=4), MI (n=3), MDS (n=2). Eighteen patients (45%) were in CR1/CR2, whereas 22 (55%) had active disease at the time of transplant; 10 patients (25%) were receiving a second allogeneic transplant. The median marrow cell dose given was $3.7x10^8$/kg (range 1.3-7.8); 11 patients were prepared with Thiotepa 5 mg/kg/day x 2, Fludarabine 50 mg/$m^2$/day x 3, Busulfan 3.2 mg/kg/day x 3 and 11 patients were prepared with TBI 1000 rads in 3 fractions and Fludarabine. High dose of Cy was given at 50 mg/kg day+3, day+5. Cyclosporin and mycophenolate were given from day 0 and + 1 respectively.
Results: Three patients died before day 10 of haemorrhage and infections. One patient had autologous recovery and died with progressive disease. Of the 36 (90%) evaluable patients all engrafted with 100% donor chimerism by day +30. Hematologic recovery was complete in 33 (82%) patients. The median times to neutrophil (>500/μL) and platelet recovery (>20,000/μL) are 18 days (range, 13-30 days) and 23 days (range, 14-58 days), respectively. GvHD was scored as grade I in 13 patients (32%), grade II in 3 patients (7%) and grade III in 1 patient (2%). The incidence of grade II-III GvHD was 9%. With a median FU of 166 days (46-465), NRM is 11% and 28% in patients with CR1/CR2 or active disease (p=0.1) (Figure 1), RRD 0% *vs* 28%; p=0.01, and actuarial survival 87% *vs* 36% (p=0.008) (Figure 2). Causes of 13 deaths were: MOF (2), Adenovirus pneumonia (1), Legionella pneumonia (1), haemorrhage (2), sepsis (2) leukemia relapse (n=5).
Conclusions: Myeloablative HLA-haploidentical BMT with T cell replete bone marrow and HD-CY is associated with high rate of engraftment, low rate of acute GVHD, and NRM and encouraging survival in a setting of very poor risk patients.

**[P400]** **Overall survival : effect of disease phase at the time of transplant**

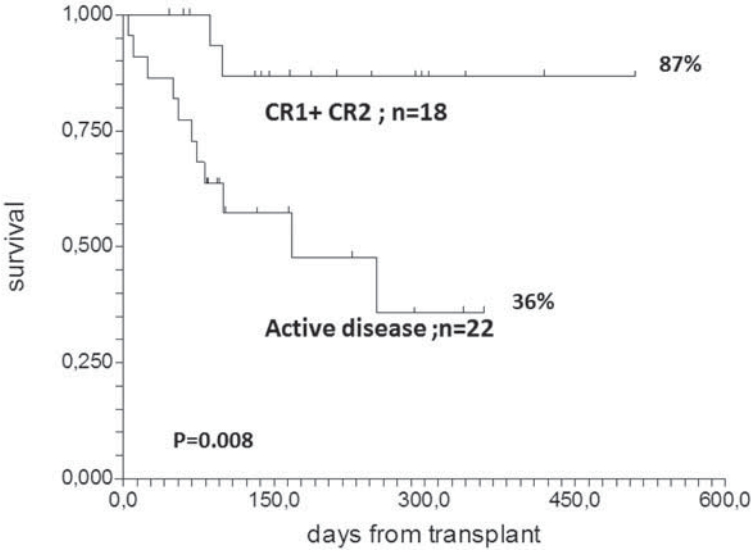


**Figure 1**

**[P400]** **Transplant related mortality and disease phase at transplant**

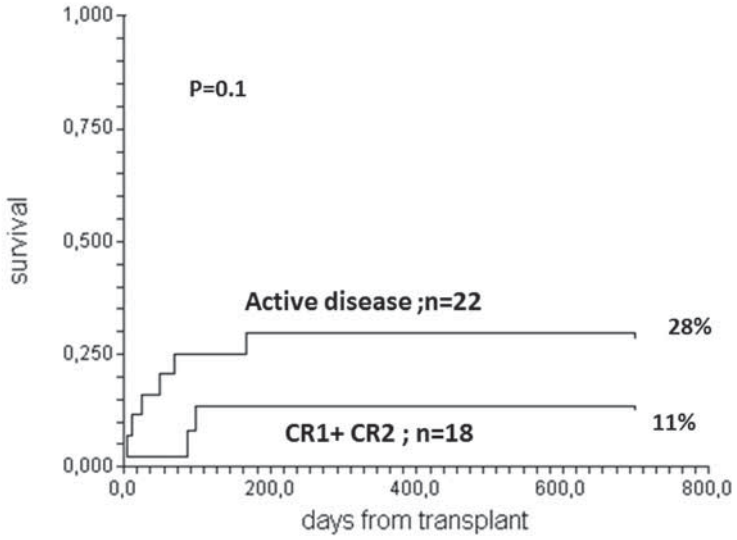


**Figure 2**

**P401**

**Killer-cell-immunoglobulin-like receptor/HLA mismatches improve survival after stem cell transplantation of patients with acute myeloid leukaemia**

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Background: HLA class I antigens are ligands for killer cell immunoglobulin-like receptors (KIRs). These receptors are expressed by NK and T-cells and thus modulate innate and adaptive immunity.
In patients with acute myelogenous leukaemia (AML), alloreactive donor natural killer (NK) cells are believed to be of significance for survival after stem cell transplantation (SCT). We tested the missing-ligand hypothesis, which indicates that alloreactivity is regulated by inhibitory KIR-receptors on natural killer NK cells and their corresponding HLA ligands on recipient cells.
Aim: To analyze the impact of KIR / HLA mismatches on post-transplant relapse, transplant-related mortality and overall survival in allografted AML-patients. We therefore performed a retrospective blinded study with patients transplanted in our centre between 1996 and 2008.
Patients and Methods: Out of a consecutive cohort of 177 AML-patients allografted in our centre between 1996 and 2008, samples from 108 donor/patients pairs were evaluable for retrospective KIR-ligand matching. The median patient age was 49 (range 20-69) years. Patients were transplanted with G-CSF-stimulated PBSC (n=103) or bone marrow (n=5) from HLA matched unrelated (n=55) donors or family related donors (n=53). KIR typing was performed as previously described. Patients were categorised according to their HLA inhibitory KIR ligand group C1, C2, Bw4, A3/A11 and presence or absence of KIR.
Results: The actuarial overall survival (OAS) of all 108 evaluable patients at 5 years is 45%. A total of 60 patients (55%) died due to relapse (n=39) or TRM/NRM (n=21). Patients with KIR-mm had a lower NRM (8/52 *vs.*13/56) and a lower relapse rate (16/52 *vs* 23/56). OAS and EFS were superior in patients with KIR-ligand mismatches (KIR-mm) compared to the group of patients without mismatches (OAS at 5 years: 59% versus 30%, log rank p=0.026, EFS at 5 years: 56% versus 33%, p=0.02). Patients with 2 KIR mismatches (C1 and/or C2) had even better survival compared to patients with single KIR mismatches.
Conclusions: AML patients with KIR ligand mismatch have a reduced mortality due to both lower relapse and as well as lower TRM resulting in superior eventfree and overall survival. Patients with AML benefit from KIR-ligand mismatched allografts. KIR typing would be a useful tool to be included to define optimal histo-compatibility of donor patient pairs.

**P402**

**Outcome of favourable acute myeloid leukaemia is improved following autologous stem cell transplantation in first complete remission**

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Objectives: Cytogenetic and molecular aberrations detected at the time of diagnosis are the most powerful predictors of outcome in patients with acute myeloid leukemia (AML). Translocations of the core binding factors (CBF) [t(8;21), inv(16)] and mutations of NMP1 gene are associated with favorable prognosis. However, 40-50% of patients still relapse. We have investigated the outcome of these patients based on post-remission therapy administered.
Methods: We report a single center experience with AML patients with favorable prognosis who were uniformly treated between the years 2002 and 2010. Sixty one patients with favorable AML were identified, including 19 patients with mutated NPM1/unmutated FLT-ITD, 16 patients with t(8;21) and 26 patients with inv(16) at a median age of 45(18-72) years. Induction treatment consisted of standard ‹7+3› regimen. Post-remission therapy included either 3 cycles of high-dose Ara-C(HIDAC) or HIDAC (busulphan 16 mg/kg/d x 4 days and cyclophosphamide 120 mg/kg) followed by autologous stem cell transplantation (Auto SCT).The decision to proceed to Auto SCT was based on insurance coverage. Patients who were not in CR post-induction or with post-consolidation relapse were referred for allogeneic (allo) SCT.

**[P402]**

| Post-remission treatment group | Overall survival (OS) | Relapse rate (RR) | Treatment related mortality(TRM) |
|---|---|---|---|
| Chemotherapy (n=21) | 23.8% | 57% | 9.5% |
| Auto SCT(n=22) | 81.8% | 23% | 4.5% |
| Allo SCT(n=18) | 50% | 27.7% | 22.2% |

Results: With a median follow-up of 16 months (2-93), there was no significant difference in treatment outcome between patients with different translocations/mutations (Table 1). Statistically significant improvement in OS and reduced RR were found for Auto and allo SCT versus chemotherapy [OS: Auto versus chemo; p=.0001, Odds ratio (OR): 14, allo versus chemo; p=.08, OR: 3.2] (RR: Auto versus chemo p=.021, OR:4.5, allo versus chemo; p=.06, OR:32). Treatment-related mortality (TRM) was not significantly different between chemotherapy and Auto SCT cohorts (p=0.4).
Conclusions: Survival was significantly superior in patients with CBF/NPM1+ AML receiving post-induction high-dose therapy with either autologous or allogeneic SCT, mainly due to a reduced relapse risk. This was achieved with no increase in toxicity in the Auto versus chemotherapy group. Patients subjected to allogeneic SCT due to residual/refractory or relapsed disease have an inferior outcome compared with patients undergoing Auto SCT as first-line treatment.

**P403**
**Autologous stem cell transplantation is a curative procedure in acute myeloid leukaemia: a single-centre experience from 1988 to 2010**
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Background: Despite the fact that allogeneic SCT currently offers patients with high risk AML the best chance of cure, we´ve aimed to investigate the outcome of AML patients who have undergone ASCT in our center, and the parameters that have been able to influence in relapse rate (RR), overall survival (OS) and relapse free survival (RFS).
Methods: Retrospective study in 121 AML patients who have undergone ASCT between 1988 and 2010. Analysis performed in 95 patients (50 male), by excluding 26 APL:62 patients until 1999 and 33, since 2000. Median age: 45[18-74]. Median lecocyte count:17250/µL [1000-318000]. 90% de novo AML and 92% in 1º Complete Remission.81% received 3+7 as Induction, 57% of which based on Idarrubicine (12 mg/m$^2$) and Citarabine (continuous 100 mg/m$^2$). 73% received, at least, 2 courses of Consolidation, combining an antracicline with low-intermediate doses of Citarabine in 74% (500 mg/m$^2$/12 h for 5 days in 77% of them). Cytogenetic risk: 64% intermediate, 29% high and 7% low. Conditioning regimen: 62% oral BuCy, 24% CyTBI and 12% iv BuCy. Stem cell source: 52% Peripheral Blood (PB). Median time from last treatment: 94 days [30-285].
Results: Median follow-up:125 months [0-216].OS at 1,3 and 5 years: 59%, 46% and 44%. RFS at 1,3 and 5 years: 60%, 49% and 48%. No relapse after 5 years. Early mortality (<day +100): 12% (9/11 from 1988 to 1999) and late mortality, 47% (34/45 because of relapse).Secondary malignancies incidence: 13% (haematologic: 5/6), none of them had received TBI.Multivariable analysis showed that RFS at 5 years was influenced by: disease status at ASCT (55% if 1ºCR *vs* 0% if ≥2ºCR/PR/refractory disease, p<0,0001), leucocyte count at diagnosis (p<0,0001, without a significant cut-off), ethiologic classification (53% if "de novo"AML *vs* 19% if secondary AML, p=0,002) and age of recipient (60% if ≤40 years old *vs* 45% if older, p=0,031). Stem cell source, conditioning regimen or cytogenetic risk had no significance in univariable analysis. Relapse was influenced by the presence of minimal residual disease (MRD) at time of ASCT (p=0,03, with available data in 47, since 1997) and by a CD34 cells count >3x10$^6$/kg (p=0,04, with available data in 49, only if PB as stem cell source).
Conclusions: ASCT is an effective procedure of cure in AML patients (global RFS of 48% at 5 years), even in high cytogenetic risk(38% with no significant difference),offering its best outcomes in young patients diagnosed of de novo AML without hyperleucocytosis, who have undergone the transplantation in 1ºCR since 2000.

**P404**
**Allogeneic stem cell transplantation in 192 acute myeloid leukaemias: a single-centre experience from 1982 to 2010**
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Background: Giving the fact that allo-SCT currently offers patients with high risk AML the best chance of cure, we have aimed to investigate the outcome of AML patients who have undergone allo-SCT in our center, and the parameters that have been able to influence in relapse rate (RR), overall survival (OS) and relapse free survival (RFS).
Methods: Retrospective study in 192 AML patients who have undergone allo-SCT between 1982 and 2010. Analysis performed in 171 patients (85 male) by excluding 21 APL: 65 patients until 1999 and 106, since 2000. Median age: 37 [18-74]. Median lecocyte count: 13400/uL [470-250000]. 82% de novo AML. 87% in morfologic complete remission (70% 1ªCR). 14 with previous SCT. Cytogenetic risk: 55% intermediate, 34% high and 11% low. Conditioning regimen was ablative in 162 patients: 36%CyTBI, 31%BuCy and 30%BuFlu. Donor: 76% related (95% matched) and 24% unrelated (64% matched). Stem cell source: 85% bone marrow. Graft versus host disease (GvHD) prophilaxis: based on Ciclosporine in 150 patients (88%). Median time from last treatment: 73 days [12-268].
Results: Median follow-up: 61 months [1-317]. OS at 1, 3 and 5 years: 57%, 44% and 40%. RFS at 1, 3 and 5 years: 62%, 50% and 45%. Early mortality (< day +100): 26% (43% until 1999 and 15% since 2000, p<0,0001). Late mortality: 27% (basically relapse). Cumulative relapse incidence at 5 years: 35%. Secondary malignancies incidence: 5%. Multivariable analysis showed that Transplantation Related Mortality (TRM) was influenced by: year of allo-SCT (OS at 5y of 49% if 2000-2010 *vs* 28% if 1982-1999, p<0,0001), late engraftment (p=0,002) and severe aGvHD (OS at 5y of 45% if no evidence/gradeI-II *vs* 25% if grade III-IV, p<0,071). Donor type and conditioning regimen lost its univariable analysis significance. No difference was found in case of HLA and ABO discordance or donor/recipient CMV status. Multivariable analysis also showed that RR and RFS at 5 years was influenced by: disease status at allo-SCT (50% if 1ºCR *vs* 0% if ≥; 2ºCR/PR/refractory disease, p<0,002)

and cGvHD (67% if present *vs* 41% if absent, p=0,035). Cytogenetic risk losts its univariable analysis significance. No diference was found in case of ethiology or stem cell source.
Conclusions: Allo-SCT is a curative procedure in AML (global RFS of 50% at 3 years), specially when disease is under control and patient develops chronic GvHD. In the last decade, there have been important improvements in the procedure which have led to a significant decrease in TRM, and consequently, a significant increase in OS.

**P405**

**I.V. busulfan and fludarabine as conditioning regimen therapy for autologous stem cell transplantation in patients with acute non-lymphoid leukaemia**

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Introduction: As previously described (EBMT, Paris 2011), at our Center we use Busulfan and Fludarabine (BuFlu) as conditioning regimen to autologous transplant (ASCT) for patients with Acute Non Lymphoid Leukaemia (ANLL) lacking an HLA matched donor or ineligible to allogeneic transplant. In this study we updated the survey of this setting of patients with new cases. Aim of the study. To assess the safety and efficacy of combination of chemotherapy with i.v.Busulfan (Bu) and Fludarabine (Flu) in a setting of patients with ANLL and ineligible to allogeneic transplant.
Patients and Methods: From June 2008 to November 2011, we used a conditioning regimen with Flu (120 mg/sm) and myeloablative dose i.v. BU (12.8 mg/bw) (BuFlu) in 21 consecutive patients (9 females and 12 males; median age: 39,9 years, r. 6-59 yy) with ANLL who received ASCT. The study protocol was approved by the local Ethics Committee and all patients signed an appropriate informed consent. At time of transplant, disease status was: 18 patients in first complete remission (CR), 3 in second CR. Patients were classified at high risk (30% of evaluable patients) when the white blood cell count at diagnosis was higher that $30\times10^9$/l and/or cytogenetic and/or molecular status was unfavorable and/or when ANLL was secondary to a myelodysplastic syndrome; otherwise they were considered at standard risk. The source of hematopoietic stem cells was in all cases peripheral blood. A median number of CD34+ cells 4x10E6/Kg (r. 2-7.4) were infused.
Results: All patients engrafted. Most patients did not need red blood cells (47,6%) and platelets (52,3%) transfusion therapy. Late mucositis with median onset 9 days (8-11 dd) after the stem cell infusions (28,5% WHO 3-4) and fever (90,4% WHO 2) were the most important complications observed during the aplasia. The overall median time for absolute neutrophil count (>0.5x10E9/l) was 16 days (r.12-20 dd) and for platelet count (>30x10E9/l) was 15 days (r.12-22 dd). The median duration of hospitalization was 28 days (r.23-34 dd). At median follow-up of 16.5 months (r. 1-41 m.), TRM was 0% while the OS and DFS was 83.1% and 82%, respectively. All relapsed patients belonged to high-risk group. Three patients died for relapse disease.
Conclusions. Our data suggest that combination of BuFlu is a safe conditioning regimen for ANLL patients. It's necessary a longer follow-up to assess the efficacy of this therapy but these preliminary results are encouraging.

**P406**

**Autologous stem cell transplantation in AML. Long-term outcome of 406 patients from a single centre**

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We describe the long-term outcome of autologous hematopoietic cell transplantation (HCT) in 406 non-M3 AML patients, treated at our Institute from 1981 to 2011 and retrospectively identified by the EBMT Registry. Median age was 32 years (1-73) and M/F ratio 229/177. Disease status at transplant was CR1 in 289 cases, CR2 in 75, advanced disease in 42. Autologous rescue was provided with bone marrow (BM) (66%) or peripheral blood stem cells (PBSCs) (34%). Overall survival (OS) at 20 years after HCT was 46% and 40% for patients receiving HCT in CR1 and CR2 respectively (p: n.s.), with a median survival time of 165 and 30 months from transplantation.
Among patients transplanted in CR1 (n. 289) the conditioning regimen mostly employed was Bu-CY (n. 184), while the remaining received BAVC regimen (n. 80) or other schedule (n. 25). The BM/PBSC ratio was 170/119. Engraftment was obtained in 95% of patients. Leukocyte engraftment times were fastened with the use of PBSC(median 14 days, range 7-33) compared with BM (median 26 days, range 7-99).
Cytogenetic/molecular data were available for 56% of the patients in CR1; of these, at diagnosis 26% had good risk features , 68% had standard risk features and 6% had poor risk features. At 20 years, actuarial OS and disease-free survival (DFS) estimates are 46% and 45% respectively. The median follow up for survivors is 114 months (range 2-274). The non-relapse mortality rate is 27%.
Multivariate analysis showed a statistically significant impact of conditioning regimen, number of induction cycles to achieve CR and cytogenetic/molecular good risk features on the cumulative incidence of relapse. No effect of stem cell source was documented in this analysis.
Univariate analysis comparing DFS of patients with favorable biologic features with those with standard/high risk features showed a 10-year DFS of 79% *vs* 51% (p: 0.006).
This study provides long-term follow up data in a large series of AML patients treated at a single centre, and supports the observation that long-term survival is achievable in about 1/2 of patients overall and about 2/3 of patients with good risk biological features. In particular, the promising results obtained in these latter group of patients seem to identify a selected population of Ist CR AML patients who are likely to benefit from autologous transplantation.

**P407**

**Autologous haematopoietic stem cell transplantation after myeloablative regimen with oral or intravenous busulfan in acute myeloid leukaemia – a single-centre experience**

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Introduction: The most popular conditioning regimen before autologous haematopoietic stem cell transplantation (AHSCT) is BuCy with oral busulfan and intravenous cyclophosphamide. The oral administration of busulfan is associated with difficulties in predicting and assessing dose delivered for pts, what is a main cause of toxicity on one hand and insufficient effect on the other hand. The intravenous form of busulfan is more effective and has more favorable toxicity profile. The aim of this report is an assessment of safety and effectiveness of both busulfan forms in AHSCT in pts with AML.
Patients and Methods: From 1997 till 2011 in Department of Haematooncology and Bone Marrow Transplantation in Lublin there were performed 21 AHSCT in pts with AML (11 F and 20 M).

The peripheral HSC mobilised after chemotherapy (HDAra-C) and G-CSF has been the source of the stem cells in all cases. The conditioning regimen was BuCy in all pts. Oral form of busulfan was applied in 14 pts (group A) and intravenous form (busilvex) in 7 consecutive last pts since 2008 (group B). The median CD34+ cells infused after myeloablative schedule was $3.71x10^9$/kg in group A and 3.69 in group B.
Results: The median time for ANC recovery (>$0.5x10^9$/L) was in group A and B: 16 and 11.5 days, respectively. The median time for PLT recovery (>$20.0x10^9$/L) was 17 days for group A and 16 days for group B. Infectious complications were developed in 10 pts from group A (mucositis – 9, neutropoenic fever – 7, sepsis – 2) and 2 from group B (mucositis – 1, neutropenic fever – 2). Treatment related mortality was 4.8% (1 pt from group A with sepsis in aplastic period). The period of hospitalization was 33 days in group A and 30 days in group B.
There were 7 relapses in group A and 1 relapse in group B; 7 pts from group A are alive and they are still in CHR; all pts from group B (7) are alive and all are in CHR. The median time of survival and survival without relapse in group A is 2.9 and 2.8 years, respectively. These parameters in group B are following: median survival – 1.2 years and median survival without relapse – also 1.2 years. The OS and DFS in group A were both 50% but in group B were 100% and 85.7%, respectively.
Conclusions: The comparison of oral and intravenous form of busulfan as component of myeloablative schedule before AHSCT in pts with AML shows high effeciveness and more favorable toxicity profile of busilvex, especially with significant reduction of severe mucositis.

**P408**

**The impact of Flt3ITD and NPM1 mutation in adult patients with acute myelocytic leukaemia autografted in first remission**

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The prognosis of acute myeloid leukemia (AML) harboring the FLT3 internal tandem duplication (FLT3/ITD) is poor with chemotherapy. However, it remains uncertain whether the FLT3/ITD impacts outcome after autologous stem cell transplantation (ASCT).
We analyzed 357 AML patients autografted in first remission (CR1) between January 2000 and December 2009. 258 patients were ITD-negative, and 99 were ITD-positive. For 203 patients, we had information regarding the existence (88) or absence (115) of nucleophosmin (NPM1) gene mutation. ITD-positive patients had higher white cell counts at diagnosis (54 *vs* $12.5\times10^9$/l; p<0.0001) and more slow remitters (22% *vs* 11%; p=0.04). Interestingly, ITD-positive patients more frequently had NPM1 mutations (59% *vs* 40%; p=0.03). Leukemia-free survival (LFS) was lower in ITD-positive patients (34±5% *vs* 52±4%; p=0.001), and relapse incidence higher (58±5% *vs* 42±4%; p=0.002). Non-relapse mortality was similar (8±3% *vs* 6±1%; p=0.7). The only unfavorable prognostic factors were failure to achieve CR with one induction course and the presence of an ITD. When studying the groups segregated by FLT3/ITD and NPM1, only the FLT3/ITD-positive, NPM1 mutation-negative group had a lower LFS (p=0.03). Although FLT3/ITD when isolated is unfavorable, adult patients with AML, FLT3/ITD, and a NPM1 mutation may benefit from high-dose consolidation and ASCT in CR1.

**P409**

**The relapse risk of AML patients undergoing autologous transplantation correlates with the stem cell mobilising potential**

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Background: Autologous stem cell transplantation is widely used to consolidate first remission in patients with AML. An important modification in the last decade has been the transition of the stem cell source from bone marrow to peripheral blood. Whereas this shift has facilitated the collection procedure, it may also imply negative consequences, such as the recruitment of leukemic cells in the graft by the mobilization procedure ultimately leading to relapse of the disease. Therefore, the stem cell mobilizing capacity of AML patients is considered controversial, since both leukemic and normal progenitors are CD34+ and can be concomitantly mobilized.
Methods: We determined the prognostic significance of the level of circulating CD34+ cells at the day of autologous stem cell collection in 78 consecutive AML patients in first remission in a single academic center. Patients were stratified into two groups with a cut-off of 60'000 CD34+ cells/ml peripheral blood. We hypothesized that a decreased ability to mobilize stem cells reflects a chemotherapy-induced reduction in the number of normal and leukemic stem cells and that this might ultimately translate into a more favorable outcome in AML patients with less than 60'000 peripheral CD34+ cells.
Results: We observed that patients mobilizing more than 60'000 CD34+ cells per ml had, in fact, both shorter overall survival (P = 0.0274) and time to progression (P=0.0014). This was mainly caused by an increased risk of relapse in AML patients mobilizing more than 60'000 CD34+ cells/ml. Patient characteristics at diagnosis did not differ between the two groups. High levels of CD34+ cells were an independent marker for both shorter overall survival and time to progression in a multivariate analysis that included sex, age, leukocytes and LDH at diagnosis, cytogenetics and the molecular mutations in the FLT3, CEBPA and NPM1 genes.
Conclusion: Our results indicate that in AML patients undergoing autologous stem cell transplantation in first remission relapse rate was increased and overall survival was shorter for those patients with high levels of mobilized peripheral CD34+ cells. Consequently, our data suggest that stringent guidelines should be established when autografting AML patients using peripheral blood stem cells, and that there must be particular caution against performing autologous transplantations in AML patients with high levels of circulating peripheral CD34+ cells.

**P410**

**Allogeneic stem cell transplantation for advanced acute myeloid leukaemia: a single-centre experience**

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Patients with acute myeloid leukemia (AML) who are refractory to induction therapy or relapse after remission have a 1 year survival probability lower than 30%. Because of this dismal prognosis, in these patients allogeneic HSCT is considered the recommended therapy.
We retrospectively analyzed 78 patients with relapsed (n=38), primary refractory (n=34) or untreated (n=6) AML who underwent allogeneic HSCT at our Institution between 2002 and 2011, to verify outcome and to identify factors that can affect long term outcome.
Myeloablative conditioning regimens were used in 48 patients (24 siblings, 24 MUD): BuCy in 43 cases, TBI-based regimens in 5 cases. Thirty patients (18 siblings, 12 MUD) received reduced-intensity conditioning.

Acute GVHD developed in 37 out of 78 patients (47%), with similar incidence in MUD (19/36, 53%) or sibling recipients (18/42, 43%) and in patients receiving BM (14/25, 56%) or PB (23/53, 43%) stem cells. Chronic GVHD occurred in 19 of the 65 evaluable patients (29%), with an identical incidence in MUD (9/30, 30%) and sibling (10/35, 29%) transplants and in recipients of BM (6/20, 30%) or PB (13/45, 29%) grafts.
With a median follow-up time of 5 years, 13 of 78 patients (17%) are alive and in CR, while 64 have died. Mortality rate was similar in the MUD (31/36, 86%) and familiar (33/42, 79%) setting. Five years overall survival was 15% (CI: 7-24%), with a trend of al longer OS in recipients of sibling HSCT (23%, CI: 9-38) than in MUD (10%, CI: 0-20). Cause of death was disease recurrence in 37 patients (58%), infection in 10 patients (16%), GVHD in 6 (9%). One-year non-relapse mortality was 35% (n=27), with infections (n=10) and GVHD (n=6) being the two most common causes.
Analyzing the clinical factors before HSCT, age <20 years (p=0.04), performance status ≥80% WHO (p=0.002), normal karyotype (p=0.03) and a full matched donor (p=0.004) were associated with a better outcome of HSCT. However, in multivariate analysis, only performance status retained its statistical significance (p=0.007). Considering post-transplant variables, in multivariate analysis only CR at recovery (p=0.05) and development of chronic GVHD (p=0.02) were correlated with a longer survival.
Our data confirm that allogeneic transplant can prolong survival in a significant proportion of extremely high-risk AML patients. Further studies are warranted to establish the best conditioning regimen and identify strategies improving graft versus leukemia effect.

**P411**

**The results of allogeneic haematopoietic stem cell transplantation after full and reduced-intensity conditioning with busulfan, fludarabine, and antithymocyte globulin in patients with acute myeloid leukaemia**

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Busulfan, fludarabine, and antithymocyte globulin as a reduced-intensity conditioning is widely used for allogeneic stem cell transplantation (SCT). We report the results of myeloablative and reduced-intensity conditioning, followed by allogenic SCT in 29 patients with acute myeloid leukemia at diagnosis. Conditioning regimen consisted of fludarabine 150-180 mg/m$^2$, busulfan 6.4-12.8 mg/kg, and antithymocyte globulin (ATG Fresenius ) 5-15 mg/kg (Flu-Bu-ATG) followed by peripheral blood stem cell allografting. The median age was 39.3 years. Among five patients with MRD+ before transplantation, 3 become MRD-posttransplant. The incidence of acute and chronic GVHD was 20% and 44% and the cumulative incidence of non-relapse mortality at 1 year and 3 years was 0% and 8.8% respectively. With the median follow-up of 16.8 months, estimated 1. and 3. year event-free survival was 70% and 65% and the overall survival was 70% and 68% respectively. Gender, age at SCT, type of donor, disease status at SCT, and complete chimerism by day +100 did not significantly influence event-free survival and overall survival. In a multivariate analysis, no presence of chronic GVHD and CD34+ dose >8x10e6/kg were significant predictors of poor overall survival.
In conclusion, Flu-Bu-ATG protocol, as a full or reduced-intensity dose regimen, can provide effective disease control with low non-relapse mortality and acceptable toxicity profile.

**P412**

**Allogeneic matched-sibling haematopoietic cell transplantation for acute myeloid leukaemia in first complete remission: comparable outcomes between Eastern Mediterranean (EMBMT) and geographically-restricted European (EBMT) participating centres**

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There has been a significant increase in allogeneic HCT activity in the WHO-designated Eastern Mediterranean area over the past decade alongside the establishment of the Eastern Mediterranean Blood and Marrow Transplantation (EMBMT) group. However, comparative outcome data with longer established centers have not been reported. The aim of this study was to compare outcomes of matched-sibling allogeneic HCT between EMBMT and European countries within EBMT from 2003-2009 among adult acute myeloid leukemia patients (pts) in CR1 using myeloablative conditioning. A total of 440 EMBMT and 1984 EBMT pts met these criteria. The 2 populations were different regarding patient, disease and transplant characteristics with notably a younger age in EMBMT pts. We were able to match 431 pts from 8 EMBMT centers with 431 pts from 27 European EBMT centers according to age (years, (range)) (EMBMT=31.4 (18-56); EBMT 31.8 (18-56), p=0.99), FAB subtype, cell source, and interval from diagnosis to allograft. There was no difference in cytogenetic risk groups or recipient gender. However, male recipients transplanted at EMBMT centers received less allografts from female donors (18% *vs.* 25% p=0.03). Also, EMBMT recipients as well as donors were more likely to be CMV seropositive (89% *vs.* 62%; p<0.001) and (89% *vs.* 52%; p<0.001), respectively. The 3-year cumulative incidence of non-relapse mortality (NRM) and relapse incidence (RI) were (NRM: EMBMT=16% *vs.* EBMT=11%; p=0.07), (RI: EMBMT=13% *vs.* EBMT 19%; p=0.053). Notably, the 3-year leukemia-free survival (LFS) and overall survival (OS) were extremely similar between the 2 groups (LFS: EMBMT=70±2% *vs.* EBMT=69±3%; p=0.97), (OS: EMBMT=74±2% *vs.* EBMT=73±2%; p=0.81). Interestingly, despite a higher putative consanguinity in EMBMT centers, the incidence of acute GVHD grade II-IV was similar between both datasets (EMBMT=30% *vs.* EBMT=25%; p=0.1). Similarly, no difference in the 2-year incidence of chronic GVHD was noted (EMBMT=40% *vs.* EBMT=46%; p=0.14).
Finally, within EMBMT centers, the 3-year OS was significantly better for more experienced centers (>50 allografts) (76 *vs.* 65%, p=0.05) likely attributed to a lower RI (11 *vs.* 23%; p=0.02) since no significant difference in NRM (p=0.7) was observed. Overall, despite the differences in socioeconomics, health resources and transplant experience, matched-sibling allogeneic HCT outcomes in emerging centers in the Eastern Mediterranean region appear similar to European EBMT centers.

**P413**

**Reduced-intensity conditioning followed by non T-cell depleted, G-CSF primed bone marrow transplantation from haploidentical donors for adult patients with high-risk acute myeloid leukaemia**

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The outcome of high-risk AML patients not undergoing an allogeneic stem cell transplant (allo-SCT) is extremely poor. The purpose of this pilot study has been to extend the use of haplo-SCT to patients aged > 55 years and/or unfit for myeloablative conditioning because of comorbidities. Materials and Methods: 15 patients with median age of 59 yrs (41-71) with very high-risk AML (resistant to 1st line therapy, MRD+ at the end of consolidation, secondary leukemia, molecular and/or cytogenetics, extramedullary disease, auto SCT failure) underwent G-CSF primed bone marrow transplant (BMT) from haploidentical family donor. Out of the 15 patients, 7 were in CR1, 5 in CR2 and 3 with active disease. Twelve of 15 patients were conditioned with a chemotherapy based regimen consisting of Thiotepa (5 mg/Kg/day on day -6), once daily i.v. Busulphan (3.2 mg/Kg/day on days -4 and -3) and Fludarabine (50 mg/m$^2$/day on days -5, -4 and -3): TBF-ric protocol. 14 patients received an identical GvHD prophylaxis consisting of pretransplant ATG combined with CSA, MTX, MMF and Basiliximab (an anti-CD25 monoclonal antibody), MMF was deleted in 1 patient with extramedullary disease at transplant. Donors (mismatched at 2 or 3 HLA loci) were primed with G-CSF at 4 mcg/Kg/d for 7 consecutive days. BM was harvested on day 0 and infused unmanipulated. Five CMV+ patients received graft from CMV- donor.
Results: The median number of total nucleated, CD34+ and CD3+ cells infused was 6.9 (4-13.2)x10$^8$/kg, 1.95 (0.74-4.8)x10$^6$/Kg and 2.8 (0.87-6.7)x10$^7$/Kg, respectively. The cumulative incidence (CI) of neutrophil engraftment was 100% at 30 days with full donor chimerism, median of 20 (16-24) days. Acute GVHD was absent or just grade I in 10 patients (66%) and the CI of III-IV grade acute GVHD was 13% (2 patients). Extensive chronic GVHD occurred in 1 (8%) out of 13 evaluable patients. For all patients, the 1 and 3-year CI of TRM was 24%; the 1 and 3 year CI of relapse was 8% and 25%. The 1 and 3-year probability of OS was 66% and of DFS 50%. Conclusions: RIC-Haploidentical transplant using G-CSF primed, unmanipulated BM is correlated with high engraftment rate, low incidence of acute and chronic GVHD, low TRM and favourable outcome in patients ineligible for conventional allo SCT.

**P414**

**Busulfan-fludarabine as myeloablative conditioning regimen in allogeneic stem cell transplantation: incidence of complications and outcome. A single-centre experience**

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Association of intravenous (i.v.) Busulfan and Fludarabine (Bu-Flu) is widely employed in conditioning regimen for Allogeneic Hematopoietic Stem Cell Transplantation (HSCT). Nevertheless safety and efficacy of these regimens are not actually well defined.
Aim of the study: Here we present our experience with the following Bu-Flu myeloablative conditioning regimen: Busulfan i.v. 0,8 mg/Kg x 4/daily from day -5 to day -2 associated to Fludarabin 30 mg/m$^2$ once a day from day -5 to day -2. Anti Thymocite Globulin (ATG) 3,75 mg/Kg at day -3 and -2 has been added in Matched Unrelated Donor (MUD) transplants.
We performed 30 HSCT (15 from MUD and 15 from sibling donor) during the last three years employing Bu-Flu as conditioning regimen. All the patients (pts) were affected by hematologic malignancy and specifically from: Acute Myeloid Leukemia: 12 patients pts, Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma: 9 pts; Myelodisplasia: 6 pts; Myelofibrosis: 2 pts; Peripheral T-cell Lymphoma:1 pts. Remissional status was: complete remission for 17 pts, partial remission for 5 pts and stable or active disease for 6 pts.
Results: 24/30 (80%) pts are still alive, 23/30 (77%) pts are in complete remission. 6/30 (18%) pts died: 4 (13%) for transplant related mortality (TRM) and 2 for relapse. We observe severe mucositis (WHO grade 3 or 4) in 12/30 (40%) pts and low-moderate mucositis (WHO grade 1-2) in 14/30 (47%) pts. Incidence of grade 2-4 acute Graft versus Host Disease (GvHD) was 5/30 (17%) pts with 2 related death and incidence of chronic GvHD was 6/30 (20%) (2 extended and 4 limited). 24/30 (80%) pts experienced fever in neutropaenia, but only 4 of them had pneumonia with 2 related deaths.
After median time of observation of 18 months overall survival (OS) is 80%, and Disease Free Survival (DFS) 77%. Median of OS and DFS is not still reached.
Conclusion: in our experience Bu-Flu regimen is safe. The major side effects are mucositis of different entity and fever. We observed a low incidence of TRM, severe infections and acute GvHD. Also DFS and chronic GvHD had a low incidence and OS is good, but the median time of follow-up is still too short for these last conclusions.

**P415**

**Allogeneic transplantation from matched and alternative donors in elderly: results in 28 patients**

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Background: The incidence of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) increases with age, being median age at diagnosis 65-70 years (y). Allogeneic (allo) stem cell transplantation (SCT) is the only curative strategy for high risk AML and MDS. However, in elderly patients (pts) an alternative donor is not considered a standard option.
Aim: To present a retrospective analysis of outcome of alloSCT from any donor source in a fit population of pts aged over 60 transplanted at our center.
Materials and Methods: Sixty-two pts older than 60 received alloSCT for high risk AML/MDS from a matched related, matched unrelated, haploidentical or cord blood donor at our Institute in period 2002-2011. We selected for this analysis those with a HCT-IC (Sorror) co-morbidity score 0-2 (N=28). Characteristics of these pts are summarized in the table.
Results: Twenty-seven pts engrafted and were evaluable at day 30 (96%). Thirteen (49%) developed aGvHD; 8 grade 1-2, 5 grade 3-4; 10 (48%) out of 21 evaluable pts had cGvHD.
Transplant related mortality (TRM) was 25% overall (7 pts), being infection and GVHD major causes of death. Variables associated with increase of TRM: PS(ECOG) over 1 *vs* 0 (50% *vs*12% at 3y, p=0.05) and aGvHD grade 3-4 *vs* 1-2 (100% *vs* 45%, p=0.02). Relapse incidence was 35% with a median time to relapse of 234 days (114-931): presence of disease at SCT was associated with a significant risk of relapse at 3y (33% *vs* 17% p=0.03) while pts with cGvHD showed a trend to less relapse.
After a median follow-up of 842 days, 15 patients (54%) are alive, 13 in complete remission (47%) and 2 (7%) currently receiving salvage treatment for disease relapse.
Estimated event free survival (EFS) at 3y is 38%, median 808 days (83-2377). Variables associated with significant worse EFS at 3y: presence *vs* absence of disease at SCT (11% *vs* 46%, p=0.02) and aGvHD grade 3-4(0% *vs* 32% p=0.01); pts with cGvHD showed a trend to better EFS. Estimated overall survival (OS) at 3y is 45%, median 945 days (83-2377).

**[P415]**

| **Gender** | | | **Donor** | | |
|---|---|---|---|---|---|
| M/F = 15/13 | | | MRD | 7 | 25% |
| | | | MUD | 6 | 21% |
| **Age :** | | | HAPLO | 13 | 46% |
| Median 64 | | | CB | 2 | 8% |
| (range 60-72) | | | | | |
| | | | **Conditioning** | | |
| **Diagnosis:** | | | treo/flu/atg/TBI | 5 | 18% |
| AML | 17 | 61% | treo/flu/atg | 14 | 50% |
| MDS | 11 | 39% | treo/flu | 7 | 25% |
| | | | other | 2 | 8% |
| **PS ECOG** *(27 evaluable)* | | | | | |
| PS=0 | 22 | 81% | **GvHD prophylaxis** | | |
| PS=1 | 4 | 15% | CSA/MTX | 18 | 65% |
| PS≥2 | 1 | 4% | MMF/Rapa | 6 | 21% |
| | | | Ex-vivo T-depletion | 4 | 14% |
| **Status @ SCT** | | | | | |
| CR1 | 8 | 29% | **Sorror score** | | |
| CR2 | 4 | 14% | Sorror 0 | 12 | 43% |
| REFR/ REL | 11 | 39% | Sorror 1 | 9 | 32% |
| upfront | 5 | 18% | Sorror 2 | 7 | 25% |
| | | | | | |

Variables associated with better OS: no presence of disease at SCT (72% *vs* 27%, p=0.03), PS=0(35% *vs* 0%, p≤0.001) and presence of cGvHD (67% *vs* 52% for 21 evaluable pts, p=0.04).
Conclusions: AlloSCT is feasible and potentially curative in AML/MDS elderly patients with HCT-IC score less than 2. Donor source did not influence the patient outcome. Moreover, among pts with Sorror score of 0-2, PS and disease status should be considered for further pts selection.

**P416**

**Outcome of high-risk and refractory AML/MDS patients receiving FLAMSA sequential chemotherapy regimen followed by reduced-intensity conditioning and allogeneic haematopoeitic stem cell transplantation**

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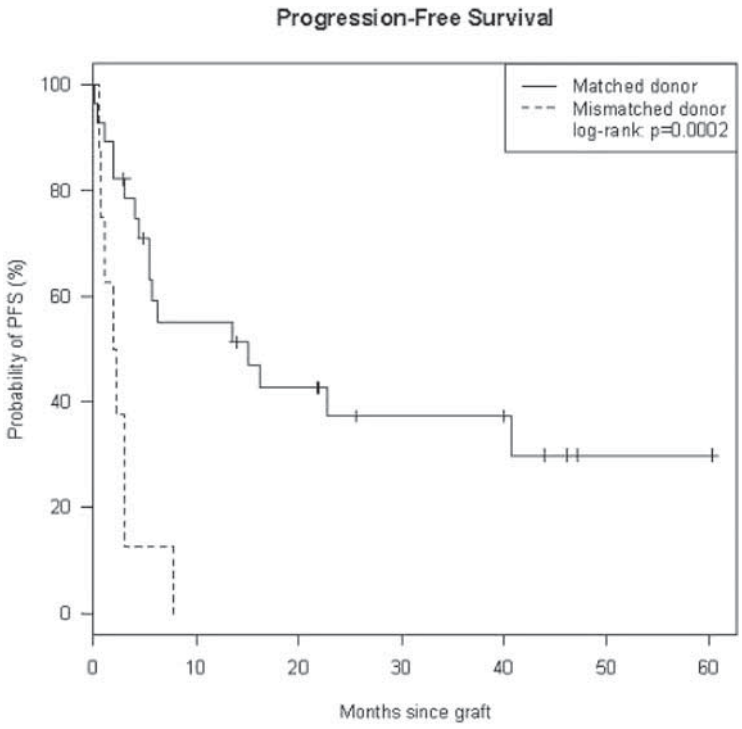
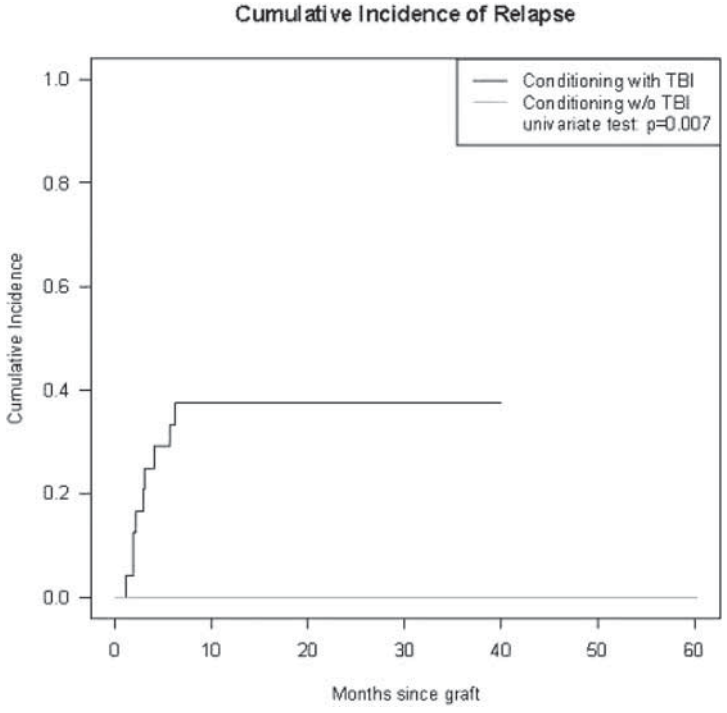
This retrospective analysis aimed to assess the outcome of 40 patients with refractory or high risk AML/MDS who received FLAMSA sequential chemotherapy. There were 30 males and 10 females with a median age of 52 years (32-66). Diseases characteristics were: progressive or refractory disease after rescue treatment for first relapse (n=21), early relapse without any further salvage therapy (n=4), and primary induction failure (n=4). The series also included 7 patients with high risk MDS and 4 patients in first CR but having a very poor prognosis. The FLAMSA regimen included Fludarabine (30 mg/m²/d), cytarabine (2 g/m²/d) and amsacrine (100mg/m²/d) from day -12 to day -9. After 3 days of rest, a RIC regimen was administered. In 28 patients, the RIC regimen included 4 Gy. TBI, ATG 5 mg/kg total dose, and cyclophosphamide 40 mg/kg in case of matched related donors, and 60 mg/kg for unrelated or mismatched donors. In the remaining 12 patients, TBI was replaced by I.V. Busulfan 3.2 mg/kg/d for 4 days. Eighteen patients were transplanted using an HLA identical sibling donor, and 22 received transplant from an unrelated donor. After allo-HSCT, 39 patients (97.5%) engrafted. In the CR group (n=4), after a median follow-up of 5 months (range, 3-31) all patients were still alive in CR at last follow-up. In the remaining 36 patients, 9 patients developed acute GVHD ≥2 with a cumulative incidence at 3 months of 18% (95%CI, 10-26). At day 90 post HSCT, 23 (64%) patients achieved hematological CR, and 14 of the 23 remained in CR at last follow-up. After a median follow-up of 6 months (range, 1-60), the 2-years probability of OS was 30% (95%CI, 17-52), and the 2-years probability of PFS was 29% (95%CI, 17-50). The cumulative incidence of relapse at 1 year was 25% (95%CI, 18-33). Interestingly, none of the patients who received Busulfan instead of TBI, relapsed. The cumulative incidence of TRM at 3 months and 1 year were 14% (95%CI, 8-20) and 22% (95%CI, 15-29), respectively.
In the multivariate analysis there was a significantly worsened PFS in patients who received transplant from a mismatched donor (HR=3.6; [95%CI, 1.3-10] p=0.01). Also, when considering disease relapse, there was a highly significant impact of the type of RIC regimen (in favour of a FLAMSA regimen without TBI (HR=0; [95%CI, 0-0] p<0.0001). A modified FLAMSA regimen incorporating I.V. Busulfan instead of TBI is likely to allow better long-term disease control, warranting prospective evaluation.

[P416]




**P417**

**Outcomes of allogeneic stem cell transplantation for secondary therapy-related acute myeloid leukaemia**

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Secondary acute leukemia is associated with a poor outcome and allogeneic hematopoietic stem-cell transplantation (HSCT) is the only potential curative therapy. We evaluated 9 patients (PTS) with secondary acute myeloid leukemia (sAML) developed after chemotherapy/radiotherapy for a previous malignancy (PM). PTS were 5 males and 4 females, median age was 43 years (range 34-62). PM was breast carcer (4 PTS), breast carcer plus gastric non Hodgkin's Lymphoma (NHL) (1 PT), osteogenic sarcoma (1 PT), lung cancer (1 PT), NHL (1 PT) and AML (1 PT). sAML occurred after a median of 24 months from PM (range 18-156). Cytogenetic analysis showed inv16 (3 PTS), t(9;22) (1 PT), t(9;11) (1 PT), del(17q) (1 PT), complex

caryotype (1 PT), normal caryotype (2 PTS). At time of HSCT 6 PTS were in 1st CR, 1 in 2sd CR, 2 with refractory disease, none had evidence of PM. Six PTS were allografted from HLA identical sibling, 2 from haploidentical donor and 1 from double umbilical cord blood (UCB) unit. As myeloablative conditioning (MAC) 3 PTS received thiotepa, fludarabine and busulfan, 2 PTS cyclophosphamide and busulfan, 1 PT thiotepa and fludarabine, 1 PT fludarabine and busulfan. Two PTS received a reduced intensity conditioning (RIC): cyclosphosphamide, fludarabine and busulfan and cyclosphosphamide, fludarabine and TBI were used, respectively. Thymoglobulin was employed in mismatched HSCT. GVHD prophylaxis was short course MTX/cyclosporin in HSCT from matched donor; short course MTX/cyclosporin plus basiliximab and mycophenolate mofetil in HSCT from haploidentical donor and prednisone plus cyclosporine in HSCT from UCB. All patients engrafted: median time to ANC > 500/uL and PLT >20.000/uL was 18 days (range 16-32) and 21 days (range 13-170), respectively. Acute GVHD I-II grade developed in 3 PTS, chronic extensive GVHD (cGVHD) developed in 3 PTS, limited in 1. Two PTS with refractory sAML progressed and died at 3 and 5 months from HSCT. One PT with extensive cGVHD developed pneumonia and died in CR at 22 months. Recurrence of previous breast cancer was observed in two PTS (1 UCB, 1 haplo) at 12 and 22 months from HSCT, while in CR for sAML. They received salvage chemo/radiotherapy and surgery: one died from refractory PM, one is alive in CR, without evidence of PM at 28 months from HSCT. Four PTS are alive in CR at 6, 24, 41, 77 months, respectively. Our results show that HSCT is a curative option for PTS with sAML.

**P418**

**Effect of additional consolidation chemotherapy before allogeneic stem cell transplantation for acute myeloid leukaemia**

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Objectives: To assess the effects of additional consolidation therapy before allogeneic hematopoietic stem cell transplantation (HSCT) in Acute Myeloid Leukemia (AML) patients who have achieved first complete remission (CR1).
Methods: Thirty AML patients except the M3 subtype (aged 16-55 years old) given less than 2 cycles chemotherapy to achieve CR after ethics committee agreement and taking informed consent form, were randomly assigned to two groups. Fourteen patients in group A received 7+3 chemotherapy regimen (cytarabine 100 mg/m$^2$/day for 7 days+ idarubicin 12 mg/m$^2$/day for 3 days) prior to allogeneic HSCT and 16 patients in group B received 7+3 regimen followed by 5+2 (cytarabine 100 mg/m$^2$/day for 5 days+ idarubicin 12 mg/m$^2$ for 2 days). All Patients were in CR1 before HSCT. The conditioning regimen consisted of Busulfan/ Cyclophosphamide in all patients. Peripheral blood was the preferred source of stem cells. Patients received HSCT from fully matched related donors.
Results: The median age at transplantation was 33.5 years in group A and 33.8 in group B. The male to female ratio was 10:4 and 4:12 in groups A and B, respectively. The median time to neutrophil and platelet recovery was 15 and 20 days in group A and 13 and 17 days in group B(Pv: 0.18, 0.32). Acute Graft- versus- Host Disease (GvHD) was more frequent in group A (11 *vs.* 9 patients), which was not statistically significant. Two relapses occurred in group B, while no relapse was observed in group A. Two patients died of aGvHD and CMV infection in group A. The causes of death among three patients who died in group B were relapse in two cases and GvHD in one case. The mean onset of aGvHD was 16.5 days in group A and 18.7 in group B. The median follow-up time was 191 days. The 6-month disease – free survival (DFS) and overall survival (OS) were both 84.4% (SE: 10.2%) in group A. The 6-month DFS and OS were 75 % (SE: 12.7%) and 73.1% (SE: 13.6%) in group B, respectively.
Conclusion: Although adding one course consolidation chemotherapy before HSCT surprisingly reduced the cumulative incidence of aGvHD, no improvement in DFS and OS was observed. As the results of the study were obtained regarding the small number of patients and short- term follow up, it seems that adding one course of treatment due to causes including drug toxicity and delay in transplantation is not appropriate. So, it should be avoided if there is any center that can provide facilities for transplantation in short time.

**P419**

**Early allogeneic haematopoietic stem cell transplantation in acute lymphoblastic leukaemia patients**

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Objectives: to evaluate the early transplantation outcome in Acute Lymphoblastic Leukemia (ALL) Patients. Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) is curative for many hematologic and non- hematologic malignancies, but the best time of doing is still controversy.
Methods: In this phase II non- randomized open labeled clinical trial with historical control group, from 2008-2011 newly diagnosed 16-50 years old ALL patients, after ethics committee agreement and taking informed consent form, divided in two groups. Eighteen patients who do not have any central nervous system or testicular metastasis and other complications assigned to receive cytoreduction (Vincristine+ Dexamethasone) and transplantation after disease stabilization at day 15 without intent on complete remission. Seventy two patients who did not meet the criteria assigned to control arm and give conventional conditioning regimen (Busulfan+ Endoxan) and then underwent HSCT after first complete remission (CR1), all from peripheral blood source. They were taken intrathecal Cytarabine, Methotrexate and Hydrocortisone for CNS relapse prophylaxis.
Results: The median age was 21.5 (range: 16-33) in the study (cytoreduction) and 22 years (range: 3-49) in control arm, respectively. The median waiting time from diagnosis to HSCT was 56 days (range: 28-123) in study and 217 days (range: 45-708) in the control group. Fourteen (77.7%) patient of study group transplanted at CR1. The median follow-up time was 15.5 and 10.2 months in the study and control arms. Relapse occurred in 2 (11.1%) and 6 (8.3%) patients of the study and the control arms, respectively. Five patients (29.4%) of the study arm and 10 patients (13.5%) of the control arm were died. The causes of death were graft-versus-host disease (GvHD) and sepsis in 4 patients and relapse in 1 patient in the study arm. The causes of death were GvHD in 6 patients and relapse in 4 patients in the control arm. One-year disease-free survival was 81.9% and 82.4% in study and control group, respectively (P-value: 0.532). One-year overall survival was 81.9% and 84.8% in study and control arms, respectively (P-value: 0.221).
Conclusion: Till this time of follow up, reduced induction chemotherapy followed by early HSCT without consolidation revealed no significant statistical different outcome compared with routine treatment but this conclusion due to small size of cases and short time of follow-up should interpret cautiously.

**P420**

**Prospective validation of a novel myeloablative conditioning regimen: intravenous F-BU 4 in patients with acute myeloid leukaemia: comparative study with the standard conditioning regimen BU-CY2**

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Introduction: The goal of allogeneic stem cell transplant (HSCT) is inducing a graft versus tumor immune effect, that's why the

choice of the conditioning may have an impact on the overall outcome of the transplant. In fact, fully myeloablative conditioning regimens can destroy tumor cells effectively, but can also cause greater morbidity and mortality. IV F-BU4 conditioning (BF) prior to allo HSCT in adult patients (pts) with AML seem an alternative to standard IV BU-CY2 regimen. We compare results with a retrospective study, about 160 AML pts, undergoing an HSCT, with the standard BU-CY2 or BU-CY2-VP16 regimen (BC).
Material and Methods: Between February 2008 and March 2011, 101 AML pts received an allogeneic HSCT, with FLU (200 mg/m$^2$) during 4 days and once daily iv BU (12,8 mg/Kg) conditioning (BF). The median age is 32 years (18-62), the sex ratio is 1,7. Eighty seven HSCT were performed in first remission (86,1%), 8 in second remission (7,5%) and 6 in relapse. The median time from the diagnosis to transplant is 6 months (2-11). For the 160 pts who received the standard regimen between September 1999 and April 2010, the median age is 27 years (18-47), 146 pts is in 1st remission, 11 pts in second remission and 3 in relapse. All pts received a transplant from a related donor. GVHD prophylaxis associated cyclosporine and methotrexate for all.
Results: All pts have neutropenia (with BF and BC); the duration of neutropenia is shorter with BF (9 versus 13 days) (p=0,084). Acute GVHD was observed in the same rate with the both regimen (24,3% in BC versus 30,9% in BF). The same was observed with Chronic GVHD (46,8% in BC, with 26% of extensive form versus 51,6% in BF, with 31,4% of extensive one's). The rate of relapse is not different with both of them (11,8% in BC versus 15,8% in BF). The study showed that the TRM were less with BF (14,8% versus 31,2%) (p<10-6). No veino-occlusive disease was noted with BF, versus 9,7% in BC. At November 2011, 94 pts of the BC group are still alive versus 73 pts in BF. The Overall survival (OS) in BF is 68,2% versus 58,1% in BC (0,1<p<0,2), and the disease free survival (DFS) in BF is 62,9% versus 57,6% in BC (p=0,9).
Conclusion: Intra venous F-Bu 4 is an acceptable regimen because of its low TRM and morbidity. It may well substitute BU CY2 with the aim of decreasing transplant adverse effects without compromising its efficacy. The duration of neutropenia is shorter, that reduce the duration of the hospital stay.

**P421**

**Preliminary results of an AUC intensification strategy with once daily Busilvex® in patients with AML in CR 1 undergoing allogeneic bone marrow transplant**

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Background: IV busulfan (Bu) followed by Cyclophosphamide (Cy) is part of standard conditioning therapy used prior to Allogeneic Bone Marrow Transplant (Allo-BMT). Although this myeloablative regimen yields a good tolerability and efficacy, the relapse rate in Acute Myelogenous Leukemia (AML) patients remains as high as 25 %. Therefore, we hypothesized that Area Under the Curve (AUC) intensification could improve the disease control and reduce the relapse rate in high-risk patients. Besides, Bu once daily schedule is increasingly used by transplant units and represents a real comfort and additional security for the patient, caregivers and pharmacists.
Objective: To target the upper range of the Bu therapeutic window (i.e.: AUC = 4400 – 6000 µM.min) from a once daily BuCy2 regimen in adult patients with AML.
Methods: Eligible patients were adults with AML in First Complete Remission (CR1) and candidate for a BuCy2 conditioning prior to Allo-BMT. In all patients, the Bu AUC was measured each day based on a limited sampling design (on-line analysis of 3 blood samples per day by on-site PK facilities) and a Bayesian calculation. Patients received Busilvex® 3.2 mg/kg on the first day and subsequent daily Bu doses were adjusted (on the previous day AUC result) in order to achieve the middle of the therapeutic area (AUC = 5200 µM.min).
Results: Preliminary PK results on 21 patients are available. After dose adjustment, most of patients reached the desired AUC range on a daily basis. The mean AUCs were 5168, 5839 and 5019 µM.min on days 2, 3 and 4, respectively. Based on the cumulated AUC over the 4 days of Bu treatment, 19 of 21 patients (90 %) achieved the targeted window.
Short term clinical outcomes were available in 14 patients (19.3-51.9 years, KPS: 90-100%). The median time to onset of neutropenia was 3 days. Median time to reach neutrophil and platelets engraftment was 19 days and 15 days, respectively, and there was no primary graft failure. The hepatic tolerance was excellent with no VOD and no treatment related mortality up to day+28.
Conclusions: This study showed that AUC intensification based on a PK-based dose adjustment is feasible and enables to target the upper area of the therapeutic window. High and controlled exposures to Bu following once daily doses were achieved in AML patients without any major safety concerns. Long-term outcomes are needed to conclude on this new therapeutic option benefit in AML patients at high risk of relapse.

**P422**

**Outcome of haematopoietic stem cell transplantation for rare types of acute myeloid leukaemia: a nationwide study of FAB-M6 and M7**

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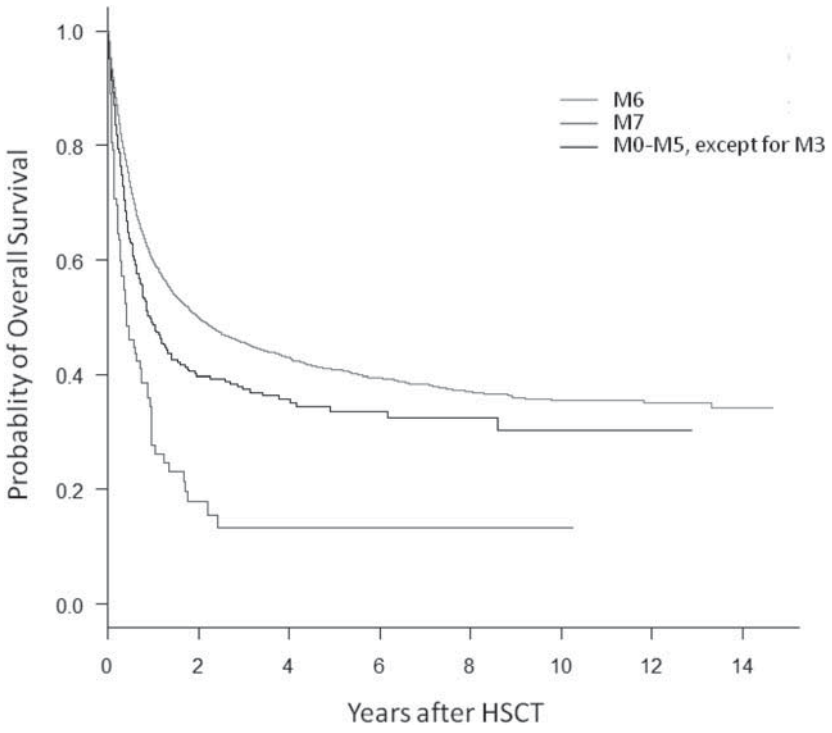
Introduction: Acute erythroid leukemia (M6: FAB classification) and acute megakaryoblastic leukemia (M7) are rare, comprising <5% of acute myeloid leukemia (AML). These two diseases have a similar morphology and shared gene expression patterns, thus suggesting a common origin. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered for these patients due to their poor prognosis; however, the effect of HSCT on the prognosis of M6 or M7 patients remains unclear because of their rarity. A retrospective study was conducted to examine the HSCT outcomes of M6 or M7 patients, using the data from the Japan Society for Hematopoietic Cell Transplantation Data Registry.
Methods: This study identified de novo AML patients categorized as M6 or M7 who underwent first allo-HSCT between January 1996 and December 2007. M0-M5 (except for M3) patients were also selected for a matched-pair analysis, matched for age, disease status at HSCT and graft source.
Results: there were 303, 80 and 5452 patients with M6, M7 and the M0-M5, respectively; and 303 and 160 of the M0-M5 patients were selected for the matched-pair analysis by a 1:1 and a 1:2 matching ratio, respectively. The 5-year overall survival

[P422]




(OS) of M6, M7 and M0-M5 patients was 34%, 13% and 41%, respectively; the OS of M7 patients was significantly lower than that of M6 patients and M0-M5 patients (P<0.01). No significant differences were observed between the M6 patients and the paired M0-M5 patients in the 5-year OS and progression-free survival (PFS), 3-year cumulative incidence (CI) of relapse and 3-year CI of non-relapse mortality (NRM); however, the 5-year OS, PFS and the 3-year CI of relapse were significantly worse in the M7 patients than those of the paired M0-M5 patients (5-year OS, PFS, 3-year CI-relapse and CI-NRM of the M7 patients and the paired M0-M5 patients was 12% and 34% (P<0.001), 17% and 33% (P<0.01), 47% and 33% (P<0.05) and 36% and 35%, respectively). A proportional hazards model showed the disease status at HSCT, a donor who was positive for cytomegalovirus antibody and patients' age ≥ 50 years in the M6 patients and the disease status at HSCT in the M7 patients to be the significant variables affecting the OS.
Conclusion: AML-M6 and M7 might therefore be clinically different disease entities with different prognostic factors. The use of centralized data thus makes it possible to perform analyses with statistical methods, even for rare diseases such as M6 and M7.

**P423**
**Transplant outcome according to the cytogenetic mosaicism in adult adverse-risk patients with acute myelogenous leukaemia**
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Background: Karyotype analysis in acute myeloid leukemia (AML) is one of the known powerful prognostic factors for complete remission (CR), relapse, overall survival (OS) and disease free survival (DFS). In these days, with development of G-banded technique, we are facing a number of miscellaneous cytogenetic abnormalities. Cytogenetic mosaicism at diagnosis is considered as one of the important characteristics in expression of phenotypic manifestations, but it has not been focused as a prognostic factor in AML because of emerging molecular biology and several mutation studies.
Materials and Methods: In this single center retrospective study, intermediate-risk cytogenetic mosaicism (e.g. 46,XY or 46,XX, trisomy 8 is accompanied by the portion of more than 10%) in AML patients of adverse-risk karyotype could be identified as a factor that can make the originally poor prognosis to the better one for OS and DFS. Adverse-risk and undefined karyotypes were based on the NCCN guidelines 2011. Clinical correlates and prognostic relevance of mosaicism were evaluated in 163 AML patients (adverse-risk karyotypes were 69 and undefined karyotypes were 94) who all underwent induction and consolidation chemotherapies and finally, hematopoietic stem cell transplantation (HSCT). And each was divided into two groups either with mosaicism or not.
Results: Seventy patients were with more than 10 percent (e.g. More than 2 out of 20 metaphases analyzed) of the intermediate-risk karyotype mosaicism and 93 were without mosaicism. Between the two groups, age, sex, induction chemotherapy cycles to achieve CR and total chemotherapy cycles before HSCT, HSCT type and source and intensity were not significantly different (p>0.05). Multivariate analyses identified intermediate-risk karyotype mosaicism in patients with adverse and undefined karyotype significantly correlates with better OS(p=0.005) and DFS (p=0.019).
Conclusion: Combined intermediate-risk karyotype mosaicism could make better prognostic expectancy in adverse-risk karyotype patients in AML. Cytogenetic mosaicism at initial diagnosis may be a valuable influential factor to transplant outcome.

**P424**
**The outcomes of allogeneic stem cell transplantation in AML patients with monosomal karyotypes**
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Background: The prognosis of acute myeloid leukemia (AML) with monosomal karyotype (MK) was reported extremely poor.

We investigated the role of allogeneic stem cell transplantation (allo-SCT) for those with MK.
Methods: A total of 114 patients who received allo-SCT for treatment of AML were retrospectively analyzed. All patients were treated with standard induction chemotherapy with anthracycline and cytarabine. Cytogenetic abnormalties were grouped according to recently published MRC criteria and MK was defined as at least two autosomal monosomies or one monosomy plus one or more structural abnormality.
Results: Thirteen patients had favorable cytogenetic risk, 78 intermediate, 11 adverse without MK, and 12 adverse with MK at the time of diagnosis. Among 12 patients with MK, 5 (41.7%) achieved CR after induction therapy, 1 (8.3%) relapsed and 6 (50.0%) refractory at the time of allo-SCT. The 2-year overall survival (OS) was significantly lower for patients with MK (17.5%) compared to favorable (76.9%), intermediate (61.0%), and adverse without MK (36.4%, p=0.017). In the multivariate analysis, those with MK was related with extremely poor outcomes (HR 6.02, p=0.008), which was independent risk factor for OS. Survival benefit was observed in MK group with chronic GVHD compared to those without chronic GVHD. The median survival was 272 days with chronic GVHD (95% CI 204-339 days) compared to 159 days (95% CI 76-172 days) without chronic GVHD (p=0.010).
Conclusion: The prognosis remained poor in patients with MK despite of allo-SCT. Innovative approaches to induce GLV effects are needed to improve SCT outcomes in patients with MK.

**P425**

**Comparison of clinical outcomes of patients with first relapsed APL re-induced with chemotherapy with ATRA or an ATO and consolidated with either an autologous SCT, an allogeneic SCTor an ATO: a multicentre, retrospective study**

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Background: Patients who had first relapsed acute promyelocytic leukemia (APL) have been reinduced with either chemotherapy with ATRA (all-trans-retinoic acid) or an arsenic trioxide (ATO). In patients who achieved complete remission, either an autologous SCT (stem cell transplantation), an allogeneic SCT or an ATO is an effective mode of consolidation therapy. The aim of the study is to investigate the comparison of clinical outcomes of patients with first relapsed APL reinduced with chemotherapy with ATRA or an ATO and consolidated with either an autologous SCT, an allogeneic SCT or an ATO.
Patients and Methods: All patients with APL in first relapse after initial treatment with a conventional ATRA-based regimen, between January 2000 and December 2009, were included in this retrospective analysis. These included patients who were treated at institutions of the member of the Korean Society of Hematology AML/MDS Working Party.
Results: Of 31 relapsing patients included who achieved second complete remission (generally after a salvage regimen of ATRA combined with chemotherapy), 12 (38.7%) received allogeneic SCT, 6 (19.4%) received autologous SCT, 4 (12.9%) received ATO, and 9 (29%) patients received ATRA-based chemotherapy as consolidation therapy. Median follow-up duration was 57.7 months. Median time from initial diagnosis to first relapse was 23.0 months (range, 5.5-69.2). Overall survival, relapse-free survival, and event-free survival did not differ significantly according to consolidation therapy, respectively. However, time from initial diagnosis to first relapse was the only significantly affect OS and EFS (P=0.044 and P=0.043), respectively.
Conclusion: In this retrospective analysis for small studied population, there was no significant difference in clinical outcomes after consolidation treatment with either an autologous SCT, an allogeneic SCT or an ATO following remission induction with ATRA-based regimens in patients with first relapsed APL. Further study is needed with more patients for determining the optimal consolidation therapy after reinduced in patients with first relapsed APL.

**P426**

**Outcome of relapse after allogeneic stem cell transplantation in patients with acute myeloid leukaemia**

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Despite allogeneic-stem-cell transplantation (Allo-SCT) is the best treatment for high risk acute myeloid leukemia (AML), relapses remain a major cause of death after Allo-SCT. There is no standard of care for these patients. We retrospectively analyzed 54 consecutive patients with AML relapsing after Allo-SCT between 2000 and 2010. The objectives were to evaluate efficacy of salvage therapies. Median age was 48 [15-69] years, 61% had been transplanted in CR1 or 2 and 80% were prepared using a RIC regimen. Thirty-two patients (59%) relapsed within 6 months after allo-SCT. Twenty-four patients (44%) received intensive salvage treatment, 17 (32%) received non-intensive chemotherapy (Low dose Cytarabine (n=7), oral Methotrexate, 6-Mercaptopurine, Etoposide and/or Melphalan (n=5), Azacytidine (n=3)), 13 (24%) received supportive care only. Among the 24 patients who received intensive salvage, 17 (71%) achieved CR, 6 failed and 1 died early. One died early (induction death: 4%) and 2 additional patients died in CR at 36 and 62 days. Sensitive disease at allo-SCT was the only factor associated with CR achievement after intensive salvage (CR: 83%; others: 33%, p=0.038). No patients treated without intensive salvage achieved CR. Median OS was 3.4 months in the whole cohort. Factors influencing OS in univariate analyses were: age < 50 (median OS: 5.1 versus 1.9 months, p=0.039) time to relapse after Allo-SCT > 6months (median OS: 7.7 versus 1.9 months, p=0.014) and intensive salvage (median OS: 7.6 versus 1.8 months, p=0.005). By multivariate analysis, only time to relapse after Allo-SCT (HR: 3.8 [1.6-9.1]) and PS at relapse (HR: 2.3 [1.1-4.4]) independently influenced OS. Median RFS of the 17 patients in CR after intensive salvage relapse was 9.4 months. After intensive salvage, 6 patients in CR were given a second allo-SCT. Four died from relapse and 2 are still alive in CR at 12 and 39 months after the relapse (3 and 7 months after the second allo-SCT).
In conclusion, our study highlights the severity of AML relapse after allo-SCT. In selected patients, salvage chemotherapy produces CR but these are short lived and unlikely to bring many patients to second transplant, the results of which remains clearly insufficient. Strategies aiming at modulating immune reactivity with anti-leukemic activity such as demethylating agents, HDAC or IMIDs have to be developed and evaluated.

**P427**

**Trends in the outcome of patients with acute myeloid leukaemia transplanted in second complete remission**

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Allogeneic stem-cell transplants (alloSCT) are potentially curative for acute myeloid leukemia (AML). Patients in second complete remission (CR2) tend to experience inferior survival compared to those in CR1.
Objectives: To retrospectively investigate the effect of pre-transplant variables on the outcome of patients transplanted with AML in CR2, and to compare different conditioning regimens.
Methods: 92 patients with AML in CR2 were transplanted between 1999 and 2010 with a sequence of myeloablative (MAB, n=63) and reduced intensity regimens (RIC, n=29) (see Table). Since 2006 a single MAB (FBT400, n=21) and single

[P427]

| | Intensity | Regimen | Median age | REL/MUD | Diagnosis to transplant ≤12 months | 2-year OS |
|---|---|---|---|---|---|---|
| Transplant period 1999-2006 (n=54) | MAB | CyTBI (n=34)<br>BuCy (n=6)<br>Other (n=2) | 39 | 18/24 | 16 (38%) | 33%<br>--<br>-- |
| | RIC | FluBu (n=7)<br>FluTBI (n=5) | 57 | 6/6 | 2 (17%) | 51%*<br>(all ages) |
| Transplant period 2006-2010 (n=38) | MAB | FBT400** (n=21) | 47 | 7/14 | 5 (24%) | 62% |
| | RIC | FBT200*** (n=17) | 62 | 4/13 | 3 (17%) | 17% |

*2 year survival of patients older than 60 years =25%
** Fludarabine 50mg/m$^2$ x4 days, Busulfan 3.2mg/kg x4 days, TBI 400 cGy
*** Fludarabine 30mg/m$^2$ x4 days, Busulfan 3.2mg/kg x2 days, TBI 200 cGy

RIC regimen (FBT200, n=17) was used. Impact on survival was studied for the following variables: Cytogenetic risk at diagnosis (SWOG), modified EBMT risk score (Hemmati *et al*, 2011), CMV status of recipients and donors and time from diagnosis to transplant. Results: Median age of all patients was 47 years (range 18-70). Median follow up was 60 months (range 11-145). Peripheral blood stem cells (PBSC) were used in 62 patients. Grafts were from related (n=35) and unrelated (n=57) donors. Overall survival (OS) of all patients did not differ for recipients of bone marrow and PBSC, grafts from related and unrelated donors, CMV status of recipients and donors and cytogenetic risk at diagnosis (63% of patients). The modified EBMT risk score did not predict survival. Patients transplanted below age 60 years had a significant survival advantage (P<0.05, HR=0.46) compared to patients ≥ 60 years. OS of patients transplanted >12 months from diagnosis was superior to that for patients transplanted within 12 months (P=0.039, HR=0.51). A comparison of the MAB regimens (CYTBI *vs* FBT400) showed a trend towards improved survival in favour of FBT400 (P=0.062, HR=0.48). RIC regimens for patients older than 60 years resulted in OS of 16% at 2 years. No significant differences were seen for RIC patients >60 analyzed by regimen. In contrast, the FBT200 regimen resulted in a 2 year OS of 71% for patients >60 in CR1.
Conclusions: The use of FBT400 suggested a trend to improved survival for patients 60 years or younger transplanted in CR2. Patients above age 60 years transplanted in CR2 demonstrated poor survival irrespective of conditioning. Time from diagnosis to transplant was identified as a prognostic indicator. Survival of patients requiring a transplant in CR2 within 12 months from diagnosis was less favorable compared to patients transplanted at a later time.

**P428**
**Peri-transplantation sorafenib for relapsed/refractory FLT3-ITD positive AML**
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Background: Activating mutations in the transmembrane tyrosine kinase FLT3 can be found in approximately 30% of adult AML and confer a poor prognosis.
In the last years, FLT3 inhibitors have been investigated in clinical trials in FLT3 mutated AML.
To date little is known about the use of FLT3 inhibitors in the peri-transplantation setting of allogeneic hematopoietic stem cell transplantation (HSCT).
Methods: Six patients with FLT3-ITD positive AML with relapsed or refractory disease on standard therapy were treated with the multi-kinase inhibitor Sorafenib (400 to 800 mg per day), one before, three after and two concomitantly to allogeneic HSCT. All patients received myelo-ablative conditioning.
Results: One patient was refractory to induction chemotherapy and received Sorafenib monotherapy consecutively, leading to complete molecular remission (CMR). He then underwent allogeneic HSCT without concomitant Sorafenib and has been in sustained CMR.
Two patients experienced an early hematologic relapse after allogeneic HSCT (2 and 4 months post-HSCT respectively), both then started Sorafenib treatment, one in combination with donor lymphocyte infusion, leading to sustained CMR.
One patient was refractory to induction chemotherapy and underwent allogeneic HSCT in disease progression. Sorafenib was started concomitantly to conditioning, leading to CMR.
One patient experienced an early relapse 3 months after autologous stem cell transplantation and received reinduction chemotherapy without response to treatment. After initiation of Sorafenib he achieved CMR and underwent allogeneic HSCT two months later, concomitantly to Sorafenib treatment. Two months post-HSCT he died of disease progression.
One patient relapsed after induction chemotherapy and underwent allogeneic HSCT in disease progression, leading to morphologic CR but persistence of FLT3 mutation. Sorafenib therapy was initiated but the patient died shortly afterwards from RSV infection. No dose-limiting toxicity was recorded.
Conclusion: Out of six patients treated with Sorafenib in the peri-transplantation setting, four remain in CMR (lasting for 28, 11, 9 and 0,5 months respectively), one died of disease progression and one of infectous complications.
We conclude that FLT3 inhibition in combination with allogeneic HSCT is a promising therapeutic approach, by combining the impact of molecular targeted therapy with anti-leucemic action of the donor immune system.

**P429**

**Treatment results of relapsed acute myeloid leukaemia after allogeneic stem cell transplantation – single-centre experience**

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Background: Relapse after allogeneic stem cell transplantation (aloSCT) for acute myeloid leukemia (AML) carries poor prognosis with limited effect of currently available treatment approaches. We retrospectively analysed outcome of patients with relapsed AML after aloSCT at our centre with the aim to evaluate the potential role of donor lymphocyte infusion (DLI), combination of chemotherapy (CHT) and DLI or 2nd aloSCT in treatment of relapsed AML after aloSCT.
Patients and Methods: From 10/2001 to 10/2011 149 pts underwent aloSCT for AML, 36 pts (24%) relapsed and 22 of them were subsequently treated for relapse. Median of age of treated pts was 45 years (range,28-63 years) and at the time of aloSCT, 12 pts (55%) were in the 1st complete remission (CR) of AML and 10 pts (45%) beyond 1st CR. 13 pts (59%) underwent aloSCT after myeloablative and 9 pts (41%) after reduced-intensity conditioning (36% with related and 64% with unrelated donor). Source of stem cell was peripheral blood in 72% and bone marrow in 28% of cases. The median of time to relapse after aloSCT was 7 months (range, 2-30 months). Treatment of relapse was assessed individually and 9 pts (41%) received DLI, 10 pts (45%) were treated with CHT and DLI and 3 pts (14%) received CHT and 2nd aloSCT. DLI were administered at escalating doses. Results: 9 pts received after relapse DLI. The median of applied doses was 2 (range, 1-4 doses) and 1 patient (11%) achieved CR, which lasts for 9 months. The median of overall survival (OS) after relapse among DLI treated pts was 3 months (range, 1-11 months). Among 10 pts treated by CHT and DLI 7 pts (70%) achieved CR but subsequently 5 of them relapsed and died, 1 patient died in CR due to GVHD and only 1 patient is alive in CR for 54 months. The median of disease free survival among CR pts was 5 months (range, 3-54 months). The median of OS after relapse among CHT and DLI treated pts was 6 months (range, 1-50 months) for all pts and 8 months (range, 5-50 months) for CR pts. 3 pts achieved after CHT CR and underwent 2nd aloSCT. 2 pts died 5 and 15 months (TRM, relapse) after 2nd aloSCT and 1 patient is alive in CR for 24 months after 2nd aloSCT.
Conclusion: In spite of relatively small number of pts our data suggest that pts with relapsed AML after aloSCT have dismal prognosis using currently available treatment approaches. In future, incorporation of earlier treatment intervention, novel drug and immunomodulation approaches could improve this poor prognosis.

**P430**

**Reduced-intensity transplantation as a part of standard treatment strategy in patients aged 60 to 70 years with acute myeloid leukaemia – single-centre experience**

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Background: Outcome of patients (pts) over 60 years of age with acute myeloid leukemia (AML) treated with intensive chemotherapy is poor. To improve treatment results reduced-intensity transplantation (RIT) was establish as a part of standard treatment strategy for pts aged 60 to70 years with AML in our centre from 2003. With the aim to evaluate transplant feasibility and the role of RIT in the treatment of pts aged 60 to 70 years with AML we analysed outcome of such pts in our centre since 2003. As a part of analysis we also tried to evaluate the role of RIT using unrelated donor.
Patients and Methods: from 1/2003 to 10/2011 AML was diagnosed in 131 pts aged 60 to 70 years. 83 pts were intensively treated and 43 pts with median of age 62 years (range, 60-68 years) with AML in 1.CR (28 pts) or AML beyond 1.CR (15 pts) underwent RIT (37% HLA identical related, 40% HLA matched unrelated, 23% HLA mismatched unrelated). Source of stem cells was peripheral blood and the median of infused CD 34+ cells was $5{,}2x10^6$/kg (range, $1{,}7\text{-}14{,}9x10^6$/kg). The conditioning regimen consisted of fludarabine (30 mg/m$^2$ for 4 days) and melphalan (140 mg/m$^2$ for 1 day). CsA and methotrexate were used as GVHD prophylaxis. Pts transplanted from related or unrelated donors did not differ for any significant variables except for younger age of donors and higher amount of infused CD34+ cells in unrelated RIT.
Results: The main reasons of impossibility to implement RIT in treatment of older pts with AML were death during remission induction treatment, severe comorbidities, non-availability of donor and refusal of RIT but 52% of intensively treated pts with AML eventually underwent RIT. All pts fully engrafted and achieved complete remission (CR). 15 pts (35%) developed aGVHD (4 pts grade III-IV) and among 36 evaluable pts 20 (56%) of them developed chGVHD (8 limited, 12 extensive). With median follow-up 28 months (range, 2-92 months) 25 pts (58%) are alive (24 pts in CR). 6 pts (14%) relapsed and 5 of them died. 13 pts (30%) died due to NRM and 4 (9%) of them till day 100 after RIT. The estimated probabilities of 3-years EFS and OS are 52% and 59% without any significant influence of donor type.
Conclusion: our data show that half of intensively treated pts aged 60 to 70 years with AML were able to undergo RIT and that RIT even in case of unrelated or HLA mismatched donor is associated with acceptable NRM and encouraging disease control of unfavourable AML.

**P431**

**Decitabine in relapsed acute myeloid leukaemia after allogeneic bone marrow transplantation**

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The prognosis of relapsed acute myeloid leukemia (AML) after allogeneic bone marrow transplantation (Allo SCT) is extremely poor. 5-aza-2'-deoxycytidine (decitabine) has been used in patients with high-risk Myelodysplastic syndrome and de novo AML with varying degrees of success.
We treated eight patients (median age 48y; range 21-68y) at our institution with relapsed AML after allo-SCT with decitabine (20 mg/m$^2$ IV days 1-5 every 28 days) and withdrawal of immunosuppressants with or without donor lymphocyte infusion (DLI) between July 2009 and July 2011. Relapse of AML after allo-SCT was defined as loss of donor chimerism and morphologic or cytogenetic relapse of primary disease. Median time to relapse after allo-SCT was 180 days (range 89-300 days). Five out of 8 patients (62.5%) developed morphologic evidence of relapse (>5% blasts; range 6%-88%) and 3 patients (37.5%) had cytogenetic relapse with falling donor chimerism (bone marrow blast <5%). Decitabine was given for a median of 2 cycles (range 1-6). Three patients received planned DLI after 2 cycles of decitabine. Five out of 8 patients (62.5%; 95% CI 24.5 to 91.5%) are still alive at the time of writing this report with a median follow up of 100 days (range 56-204 days). All surviving patients attained 100% donor chimerism (4 patients after decitabine alone and 1 with decitabine followed by DLI) after a median of 60 days (range 30-180 days) or after a median of 2 cycles of decitabine. Four out of 5 surviving patients developed graft versus host disease (GVHD). Three patients developed GVHD after 2 cycles of decitabine without DLI. Of the 5 surviving patients, 3 patients had cytogenetic relapse with mixed chimerism (<5% blasts in the marrow) and 2 patients had morphologic early relapse (Marrow blasts 5-19%) at the time of diagnosis. None of the patients (n=3) with frank relapse (>20% blasts) survived. Decitabine was well tolerated in this cohort.
In our experience, decitabine with or without DLI was effective in patients with AML in early relapse after allo-SCT. This was a retrospective chart review of a small cohort with a short duration

of follow-up. However, we are in the process of developing an algorithm at our institution whereby we propose to treat eligible patients with hyper-proliferative/frank relapse (Marrow blasts >20%) after allo-SCT prospectively with clofarabine followed by DLI whereas patients with light relapse may be treated with decitabine alone.

**P432**

**Alpha n intravenous busulfan based myeloablative conditioning regimen for allogeneic haematopoietic stem cell transplantation in paediatric ALL patients**

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Objective: Total body irradiation (TBI)-based preparative regimens have been considered the gold standard for allogeneic HSCT in children with ALL; however, there are emerging concerns about the long-term sequelae of TBI in childhood. Substituting i.v. for oral busulfan (Bu) reduces variability in drug exposure, potentially improving the safety and efficacy of the treatment. We retrospectively evaluated 42 ALL patients (male 32/ female 10) transplanted in our institution, between January 2005 to July 2011, who received allogeneic HSCT, using a myeloablative i.v. Bu-based conditioning.
Patients and Methods: The median age of the patients was 4,8 years (6 months – 17,7 years). Twenty-seven patients were in CR1, 11 in CR2, 3 in CR3, and 1 in advanced disease. Donors were: HLA-matched siblings (n=10), matched unrelated (n=29), 1-Ag mismatched related donors (n=2) and haploidentical (n=1). Twenty-seven patients received bone marrow, 11 peripheral blood stem cells and 4 cord blood. Busulfan was administrated as a 2hs, infusion every 6hs over 4 days (16 doses) in combination with Cyclophosphamide and VP-16 in 34 patients. Graft versus host disease (GVHD) prophylaxis consisted of CSA+MTX in patients receiving blood or marrow stem cells and CSA only in those who received cord blood. Anti-thymocyte globulin was added to those who were transplanted from unrelated donor.
Results: All patients but three achieved sustained engraftment. Median time to ANC>500, and platelets>20.000 was 19 days (14-29 days), and 21.5 days (12-44 days) respectively. One patient died on day 10 and another patient relapsed on day 22; both were not evaluated for engraftment. There were 10 cases of mild veno-occlusive disease, and 10 cases of hemorrhagic cystitis. Grade II-IV acute and chronic GVHD occurred in 25/42 and 6/42 patients, respectively. At median follow-up of 46,4 months (5,0 – 84,1 months), 30 patients are alive/disease free and one is alive with leukemia. Five patients relapsed and died and 6 died of transplant-related causes. The overall survival (OS) rate, relapse rate, and TRM rate were 69%, 24%, and 11%, respectively.
Conclusion: Our results are comparable to those reported with TBI-based preparative regimens and suggest that i.v. Bu may be a reliable alternative to TBI in the setting of HSCT for ALL in children.

**P433**

**Once-daily IV busulfan administration with therapeutic drug monitoring showed superior outcome compared to the conventional 4-times a day busulfan administration in BuFluVP conditioning regimen for paediatric ALL/ABL patients**

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Introduction: Conditioning regimens for pediatric ALL usually includes total body irradiation (TBI), but TBI may result in serious sequelae. Recently, a reduced toxicity myeloablative regimen using busulfan and fludarabine showed promising results, and etoposide was used in addition for its anti-leukemic effect in many studies. Busulfan has a narrow therapeutic range, and it shows high pharmacokinetic variability even when intravenous (IV) formulation is used. Due to this pharmacokinetic variability, therapeutic drug monitoring (TDM) and dose adjustment of bulsulfan have been recommended to improve the clinical outcome of hematopoietic stem cell transplantation (HSCT).
Patients and Methods: We studied patients who underwent HSCT using a busulfan/fludarabine/etoposide regimen at Seoul National University Children's Hospital from September 2005 to September 2010. Twenty patients (Bu4 group) received IV busulfan (0.8 mg/kg/dose for patients >12 kg and 1.1 mg/kg/dose for ≤12 kg, 4 times/day, d-8~d-5) without TDM and targeted busulfan (120 mg/m$^2$ for patients ≥1 year and 80 mg/m$^2$ for <1 year as the first dose, once daily, d-8~d-5) was used for 14 patients from March 2009 (Bu1TDM group).
Results: The diagnoses were ALL in 27 and ABL in 7 patients. Median age was 6.0 years (range 0.9-16.6 years). Graft failure occurred in 4 patients (20.0%) of the Bu4 group, but engraftment was achieved in all patients of the Bu1TDM group (P=0.126). Veno-occlusive disease developed in 4 patients (20.0%) of the Bu4 group and 1 patient (7.1%) of the Bu1TDM group (P=0.226). Three patients (15.0%) of the Bu4 group and 1 patient (7.1%) of the Bu1TDM group died of treatment-related mortality (TRM), and a total area under the curve (AUC) of the 1 patient of Bu1TDM group was 79,469 ug*h/L. Event free survival (EFS) of patients of the Bu1TDM group was significantly higher than that of the Bu4 group (92.9% *vs* 58.5%, P=0.050). In patients of the Bu1TDM group, the total dose of busulfan administered for 4 days ranged from 249.9 mg/m$^2$ to 523.0 mg/m$^2$ (median 403.1 mg/m$^2$) with median total AUC of 74,010 ugxh/L (range 70,815-83,160 ugxh/L).
Conclusion: Our study demonstrates that once daily IV busulfan administration with TDM showed superior outcome compared to the conventional 4-times a day administration in BuFluVP conditioning regimen for pediatric ALL/ABL patients.

**P434**

**Outcome of sibling allogeneic stem cell transplant for Philadelphia positive (Ph+) acute leukaemia with different phenotypes**

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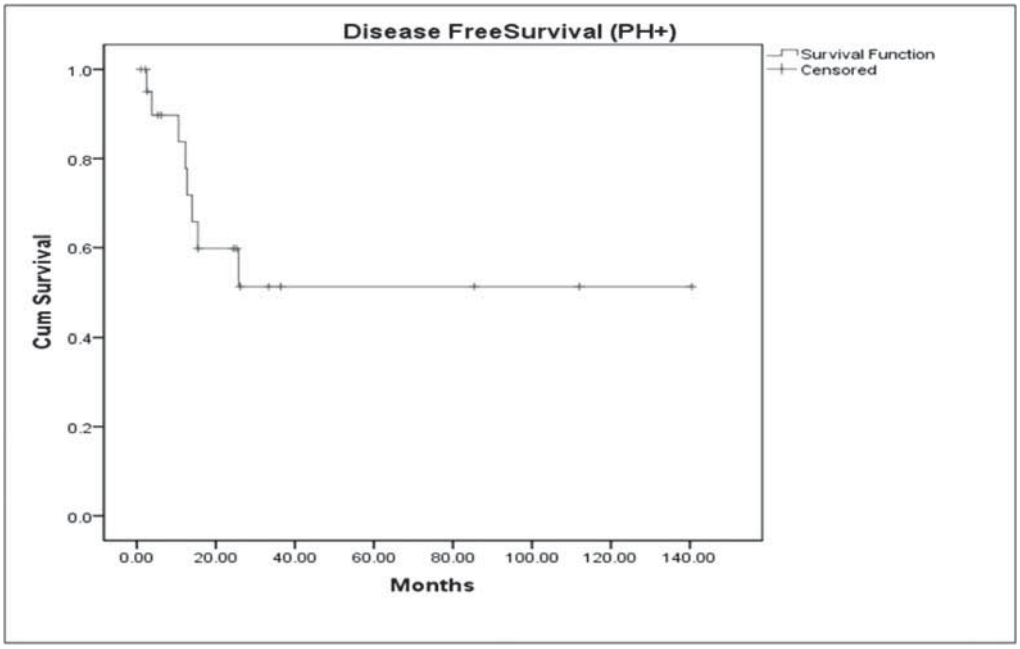
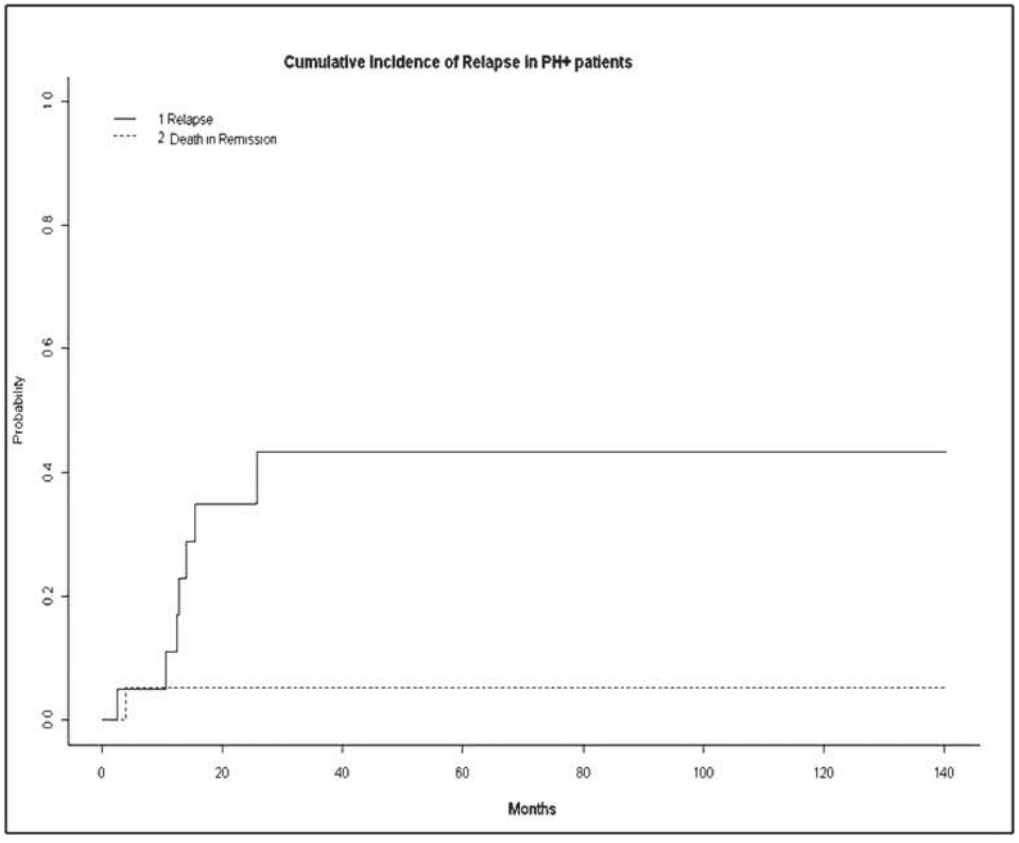
Background: PhIladelphia positive (Ph+) Acute lymphoblastic Leukemia (ALL), Acute Myeloid (AML) and biphenotypic acute leukemia (BAL) are relatively rare with young age and allogeneic stem cell transplant (Allo-SCT). Our population in Saudi Arabia is relatively young with large families and high consanguinity making myeloablative conditioning (MAC) the preferred modality and sibling donors the most common source. With increased use of tyrosine kinase inhibitors (TKIs), more patients are being transplanted. Relevant data are very limited from the region.
Patients and Methods: Between 1999 and 2010, prospectively collected data of 23 (8 females, 15 male) patients were analyzed. Median age was 27 (14-54); 12 were >14-<30. 18 had PreB-, 2 T-ALL, 2 AML and 1 BAL. 9 had additional cytogenetic abnormalities (ACA); 5 untested. All received MAC (Cy-TBI for all except BU-CY for 2 AML). GVHD prophylaxis included cyclosporine and methotrexate in all. All donors (23) were siblings; 21 were full match. 65% received peripheral blood (PB) stem cells, 20 were in CR1, 2 in CR2 and 1 in relapse. In combination with different chemo-inductions, 11 received Imatinib, 4 Dasatinib and 2 both and in 2 the use of TKI was unknown. Death in remission considered as a competing risk for relapse.
Results: Cumulative transplant related mortality (TRM) at 3.8 mowas 5.2 %. Acute GVHD occurred in 36% (95% CI 34-39%), cGVHD in 35% (95% CI33-37%). Median follow up of survivors was 24.2 mo (95% CI, 1-140).The estimated 5 years overall survival (OS) was 65.8% (95% CI, 40%-91%) without

difference by age (p 0.5). There was a trend (p=0.1) of higher rate of death in remission for those ≥30 y compared to younger ones. No impact of ACA on OS/DFS (p 0.9). There was a trend (p value 0.1) of better OS for those in molecular remission at transplantation (OS 87.5%; 95% CI, 65%-110%) compared to those who were not (OS 50%) (95% CI: 11%-89%). Molecular remission (MR) was associated with lower rate of death in remission but not significantly (p=0.3). CIR was 43.4 % (95% CI 42.9-43.9) regardless of age (p value 0.3), ACA, the molecular status at SCT, aGVHD or c-GVHD.

Conclusion: Ph+ acute leukemia of different phenotypes in our relatively younger population are mostly of pre-B phenotype. AllosibSCT offers good OS with an acceptable TRM that tended to increase with age ≥ 30 y. MR before SCT has a trend to improve survival thatneeds to be confirmed. ACA has no impact on outcome in the presence of MR.

**[P434]**



**P435**

**Allogeneic haematopoietic stem cell transplantation from mostly sibling donors for adolescent and adult patients with high-risk acute lymphoblastic leukaemia: 20-year experience**

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Background: allo-SCT from matched sibling donors remains the best option in high risk ALL. The majority of population in Saudi Arabia is young and the chance of finding sibling donor is high (68%) due to large family size and consanguinity; a setting that may help reduce transplant related mortality (TRM) and reduce graft *vs*. host disease (GVHD).
Patients and Methods: 258 (75 female, 183 male) transplanted between 1991-2011 at KFSHRC with complete data set were reviewed. 64% were PreB-, 33% T-ALL and 9(3%) unknown. Median age was 19.9y (14-54); 86% were <30y. 144 were in first remission (CR1) with high risk features: high count, induction failure, adverse cytogenetics (CyG). 77 were in CR2, 35(≥CR3). Myeloablative conditioning was mostly Cy-TBI, donors were siblings in 99% (bone marrow (BM) in 68%; peripheral blood (PB) in 31%) and were fully matched in 96%. GVHD prophylaxis included Cyclosporine and Methotrexate.
Results: At a median 40 Mo (4-254) follow up, 5y OS was 50% and was better (p 0.001) for CR1 (62.6%) *vs*. CR2 (41.8%) *vs*. ≥CR3 (16.6%); no difference (P value 0.8) with age < or >30 y, phenotype (p=0.2), CyG (p≥0.05), aGVHD(p=0.2) or cGVHD (p=0.5). DFS was 44% and was higher (57.5%) in CR1 *vs*. 34% in CR2 *vs*. 11% for≥ CR3 (p≤0.001). T- ALL had a trend of better DFS (p= 0.08). No difference with CyG (p >0.05) or age groups (p 0.4).
The rate of aGVHD was 42.9% with 5.5% 100-day mortality as a competing event. The cumulative TRM was low at 9%. cGVHD occurred in 29.5% and was higher with PB source (p=0.003). No impact of c-GVHD on OS (P 0.3). The cumulative incidence of relapse (CIR) was 34.9% (95% CI; 34%-36%). Death in remission as a competing risk for CIR was 21.5% (95%CI; 16-27); was higher (p=0.001) with aGVHD not age (p 0.7)or cGVHD(p 0.3). cGVHD had a lower CIR (27.1%) *vs*. no cGVHD(41%)(P = 0.01). Competing risk regression modeling by Fine & Gray showed that cGVHD lowered the hazard ratio (HR) of relapse to 0.58 (p 0.01). a-GVHD also lowered relapse HR to 0.55 (p=0.006) but with markedly increased HR of death in remission (HR 2.9; p≤0.001).
Conclusion: In this study, allosib-SCT in relatively young patients from families with high rate of consanguinity is associated with relatively low TRM and low cGVHD . Relapse remains the commonst cause of failure especially in ≥CR2. cGVHD improves relapse risk and a-GVHD increased death in remission 3 fold Further improvement in GVHD prophylaxis and supportive care is needed.

**P436**

**Allogeneic stem cell transplantation as a superior treatment option for adolescents and young adults with acute lymphoblastic leukaemia: a single-centre experience**

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Background: Treatment outcome of patients (pts) with acute lymphoblastic leukemia (ALL) depends on several factors: patient age, immunophenotype and clinical, cytogenetic and molecular features of the disease. Despite intensified chemotherapy, adolescents and young adults with ALL still have lower rates of survival than younger children. Majority of reports published so far indicate that these pts have a better outcome when treated with pediatric, rather than adult therapeutic protocols. Role of allogeneic stem cell transplantation (SCT) in first complete remission (CR) in this cohort of pts is still controversial.
Aim: Estimation of treatment outcome in adolescents and young adults aged 15 to 30 years with newly diagnosed ALL who were treated in our adult hematology department either with conventional approach or allogeneic SCT.
Patients and Methods: Since 1989 till 2011. a total of 82 pts, male/female 56/26, aged 15 to 30 (average age 20), with newly diagnosed ALL were treated with adult chemotherapy protocol and allogeneic SCT. In the postremission setting, pts were divided in two gruops: with „donor" (35 pts) in whom we have performed allogeneic SCT in CR1 from idenstical sibling and with „no donor" (47 pts) who were treated with conventional maintenance therapy. With respect to disease risk parameters (standard *vs*. high risk), age (15-18 *vs*. 19-30), white blood count (>30x10$^9$/l *vs*. <30x10$^9$/l), time to achieve remission (below 28 days *vs*. above 28 days) groups „donor" *vs*. „no donor" did not differ.
Results: For the whole cohort, the remission rate was 91,46%, iduction failure 8,54%, early deaths 3,65% and relapse rate 56,09%. After a median observation time of 8 years the overall survival (OS) and event-free survival (EFS) of pts who were treated with allogeneic SCT in CR1 were superior in comparisson to pts who were treated with conventional therapy (OS 42,85% *vs*. 25,53%, p<0.05; DFS 54,28% *vs*.29,78%, p<0.05). Relapse incidence was significantly lower in the group of pts with allogeneic SCT (31,43% *vs* 68,08%, p<0.05). Significantly better OS had pts with initial lower white blood count and those who have achieved remission in the first 28 days. In this cohort of pts age had no impact on the results.
Conclusion: Allogeneic SCT in CR1 is superior treatment option for adolescents and young adults with ALL in comparisson to conventional adult chemotherapy protocol, but further investigation, especially in the new target agents era is needed.

**P437**

**Non-TBI regimens for allogeneic haematopoietic stem cell transplantation in paediatric patients with acute lymphoblastic leukaemia: long-term follow-up**

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Background: The most common conditioning regimen for allogeneic hematopoietic stem cell transplantation (HSCT) consists of total body irradiation (TBI), especially in patients affected by acute lymphoblastic leukemia (ALL). However, the use of non-TBI containing regimens has been considered in recent studies.
Method: A total of 69 ALL pediatric patients aged < 15 yrs underwent HSCT in our center between 1997 and 2011. Non-TBI conditioning regimen used in this study was based on protocols approved by the center. Patients received busulfan and cyclophosphamide with or without ATG as conditioning regimen. Cyclosporine ± methotrexate was used as graft-versus-host disease (GvHD) prophylaxis regimen. Stem cell sources included bone marrow in 8 (11.6%), peripheral blood in 54 (78.2%) and cord blood (CB) in 7 (10.2%). Fifty-nine patients received allo-HSCT from HLA- matched sibling donors, 4 from other related donors, 1 from an HLA-mismatched sibling donor and 5 from unrelated donors. All the patients enrolled were assessed for the survival.
Results: Median age at transplantation was 10 years (2-14 years). Sixty three out of 69 patients received transplants in complete remission. At a median follow-up of 14 months the probabilities of two-year disease-free survival and overall survival were 65.8% (SE=6.9%) and 77.2% (SE=6.5%), respectively. Acute and chronic GvHD occurred in 47 (68.1%) and 8 (14%) patients, respectively. Relapse was significantly higher among patients transplanted in advanced disease status.

Conclusion: The study revealed an acceptable result in HSCT with non-TBI-based preparative regimens in pediatric patients with ALL. As the use of TBI- based regimens in pediatrics has a lot of side effects and is associated with general decrease in quality of their life, further comparative trials are needed to clarify the difference between two conditioning regimens in pediatric ALL.

**P438**

**Complete remission of central nervous system relapse with dasatinib 100mg/day in Philadelphia chromosome positive acute lymphoblastic leukaemia**

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Central nervous system (CNS) relapse is a catastrophic event in acute lymphoblastic leukemia (ALL), especially more common in Philadelphia chromosome positive (ph+) ALL. In patients with ph+ ALL, overall response rate and survival are significantly improved by integration of imatinib as a front line therapy but imatinib has shown poor response to the CNS relapse. Here we report a ph+ ALL patient experienced complete remission (CR) of CNS relapse with dasatinb.

The patient is 24 male referred from private clinic due to many blasts in the peripheral blood. Bone marrow study revealed he had acute B lymphoblastic leukemia and also Philadelphia chromosome was positive. After one cycle of hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone) chemotherapy he achieved complete remission. One more cycle of hyper-CVAD and imatinib 400mg/day was given before he proceeded to allo-HSCT. HSCT was performed in molecular remission from matched related donor. He has maintained remission for 10 months since allo-HSCT. At eleven months after HSCT, the patient complained headache and serum BCR-ABL turned to positive. Bone marrow study showed 99.6% donor chimerism without evidence of leukemic recurrence. Brain magnetic resonance imaging (MRI) showed prominent leptomeningeal enhancement and many leukemic blasts were detected in the central nervous fluid. Three times of intrathecal chemotherapy with methotrexate 12mg was given but blasts persisted in the CSF. Then the patient was started on dasatinb 100mg/day. Within one week of dasatinib treatment headache was improved and BCR-ABL returned to negative within 1 month of dasatinib. The patient has been maintaining dasatinib for 8 months without recurrence and specific toxicity.

Dasatinib 100 mg/day could achieve enough level to eradicate CNS leukemia. It may be due to better penetration to the CNS and high potency of inhibiting BCR-ABL. In ph+ ALL patients with CNS relapse, dasatinib should be the first choice of treatment.

**P439**

**Impact of surface CD20 expression on prognosis of adolescents and young adults with pre-B acute lymphoblastic leukaemia treated with a unified risk-stratified protocol**

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Background: There is limited, yet conflicting, data describing the impact of CD20 positivity on the outcome of PreB-ALL in pediatrics and adults using different protocols and stratifications. However, there is no available data in AYAs Patients and methods: We analyzed the impact of CD20 expression on outcome of AYAs with preB-ALL treated by CALGB-based protocol. Allogeneic stem cell transplantation (allo-SCT) was offered for those with available sibling donors with high risk features at presentation (WBC >30x$10^9$/L, poor cytogenetics), day 14 residual disease, induction on day +28 and after relapse. Patients refractory to induction were salvaged by Fludarabine plus high

**[P439]** Table 1- Clinico-pathological features of CD20 pos. vs. CD20 neg. (n: 83)

CNS: central nervous system

| | CD20 pos. N (%) | CD20 neg. N (%) | P value |
|---|---|---|---|
| Frequency | 24 (32%) | 53 (64%) | |
| Median Age (y)(range) | 18.5 (14-30) | 19 (14-29) | 0.18 |
| Female | 10 (41.7%) | 24 (45.3%) | 0.81 |
| Median WBC ( x$10^9$/L) | 7.99 (0.53- 164) | 10.9 (0.39-303) | 0.51 |
| WBC = or >30 X$10^9$/L | 8 (33.3%) | 17 (32.1%) | 1.0 |
| LDH (iu/L) | 565 (174-7418) | 704 (187-9166) | 0.42 |
| Philadelphia+ | 2 (8.3%) | 5 (9.4%) | 1.0 |
| CNS involvement | 4 (16.7%) | 5 (9.4%) | 0.45 |
| ▪ Extramedullary disease | 8 (33.3%) | 13(24.5%) | 0.34 |

Table 2. Outcome of CD20 pos. vs. CD20 neg

| | All patients | CD20 pos. | CD20 neg. | P value |
|---|---|---|---|---|
| Day 14 BM clearance | 67 (87%) | 21 (87.5%) | 46(86.8%) | 1.0 |
| CR | 75 (97.4%) | 23(95.8%) | 52 (98.1%) | 0.26 |
| CIR | 47.9% | 38.9 | 52.4 | 0.3 |
| CIR CNS | 7.8% | 11.9% | 5.2% | 0.5 |
| 5y OS | 58.9% | 68% | 53.6% | 0.2 |
| 5y DFS | 44.8% | 56.8% | 38.9% | 0.2 |

[P439] Figure -1 Overall Survival for ALL PreB stratified by CD 20(+) vs.CD20(-)

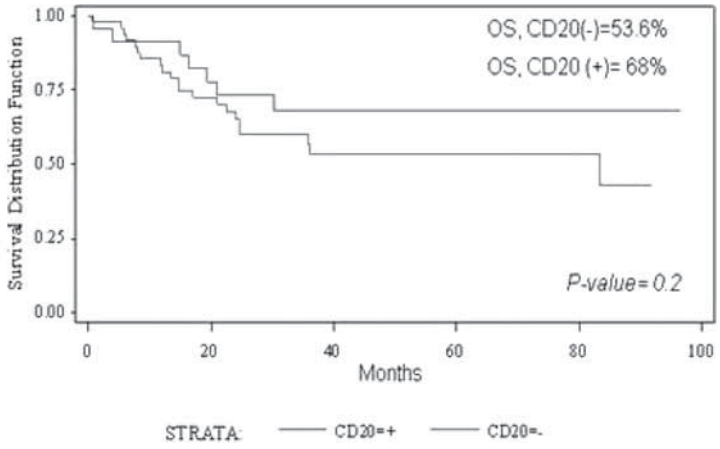
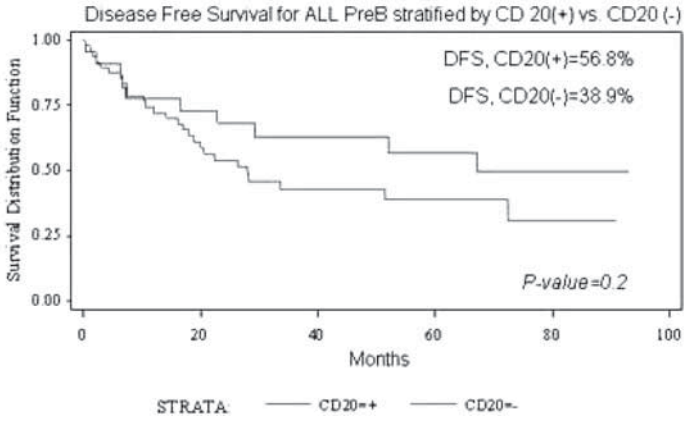



Figure -2




dose Ara-C. CD20 expression was considered positive if at least 20% of the leukemic cells express the marker.
Results: Between July 2002-June 2010 a total of 83 AYA patients were identified; 53(64%) were CD20 negative, 24 (32%) were CD20 positive and 6 (7%) were undetermined. 72 (87%) cleared blasts from BM by d14 and 10 (12%) patients required additional induction. Day + 28 Complete remission was achieved in all except 2 (2.4%) patients (CR-1=97.6 %). One induction death occurred in CR1. There were no significant differences in distribution of high risk features between the CD20 pos or CD20-neg groups as shown in the Table 1. There was no significant impact of CD20 positivity on induction mortality or early outcomes, survivals (OS or DFS) or cumulative incidence of relapse (CIR) or central nervous system (CNS) relapse. Although OS and DFS seemed better for CD20 pos yet, the difference was not statistically significant (p value 0.2 and 0.2 respectively) (Table 2, Figure 1 and Figure 2). However, more patients (69.6%) of CD20 pos. received allo-SCT compared to only 36.5% of CD20 neg. (p value 0.012).
Conclusion: In our EMRO population of AYAs with pre-B ALL and within a risk-stratified unified protocol of chemotherapy for standard risk and allo-SCT for high risk, no significant impact of CD20 expression on early outcomes (rate of clearance of leukemic blasts, CR, induction mortality) or long term outcomes (5 Y OS, DFS and CIR or CNS relapse). Allo-HCT may help balance any impact. Analysis of the impact of CD20 expression should cautiously be interpreted within the context of treatment given and the age of the population studied. Larger studies may help elucidate any meaningful impact.

**P440**

**Superiority of total body irradiation versus busulfan-based conditioning regimens prior to haematopoietic stem cell transplantation in advanced phase acute lymphoblastic leukaemia**

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The total body irradiation (TBI)-based conditioning regimen is considered the “gold standard” for allogeneic hematopoietic stem cell transplantation (allo-HCT) in acute lymphoblastic leukemia (ALL). In this retrospective study we compared the impact of TBI-and Busulfan (Bu)-based conditioning regimen on the outcome of patients (pts) allografted for ALL.
One hundred fifteen consecutive pts, aged 24 (4-58) years, underwent allo-HCT during 1990-2011. Thirty-two pts were in 1st complete remission (CR1), 20 in CR2 and 63 in advanced phase (>CR2, relapsed or refractory disease). The graft source was mainly mobilized peripheral blood, originated from 71 siblings, 44 unrelated donors (15 alternative, 1-2 ag-mismatched). TBI-based regimen (14.4Gy divided in 6 fractions over 3 days) was given in 59 and Bu-based (mainly oral, 4 mg/kg/d) in 56 pts. Cyclophosphamide (Cy) was administered at standard

doses (120 mg/kg) in both regimens. TBI-Cy was given in 19 CR1, 11 CR2 and 29 advanced phase pts. For the whole cohort of pts with a median follow-up 7(1-247) months, the conditioning regimen did not significantly affect the outcome in terms of disease-free survival (DFS): (45% *vs* 30%) and non-relapse mortality (NRM): (20% *vs* 30%). The type of donor revealed no significant impact on either DFS: 40%, 25% or NRM: 20%, 24%, for siblings and unrelated matched donors respectively).
A relatively small number of pts allografted in CR1, did not have any significant difference in DFS and relapse rate (RR) regarding the conditioning regimen (DFS: 63% *vs* 76%, RR: 24% *vs* 24%, for TBI and Bu-based regimens respectively, p=ns). NRM tended to be increased in TBI-based regimens (26% *vs* 0%, p= 0.06). In CR2, DFS and OS were similar for both regimens (39% *vs* 33% and 44% respectively). On the contrary, in advanced disease, TBI offered a superior DFS: 36% *vs* 11% and OS: 40% *vs* 17%, (p<0.05).
In a multivariate analysis only early disease phase affected DFS favorably. Age >45, graft versus host disease (GVHD) and mismatched donors increased mortality while early disease phase, younger age, the absence of aGVHD and the presence of cGVHD were proven favorable factors in terms of OS.
Overall, the type of conditioning regimen did not influence the outcome. NRM which was zero in early disease and Bu-based regimen, could be attributed to the small number of pts but should be taken under consideration.
For pts transplanted in advanced disease TBI-based regimen offered significant survival advantage with acceptable toxicity.

**P441**

**Outcome of allogeneic haematopoietic stem cell transplantation for Philadelphia chromosome positive acute lymphoblastic leukaemia in first complete remission at the era of tyrosine kinase inhibitors: a survey from the Acute Leukaemia Working Party**

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TKIs emerged as major drugs as part of Ph+ALL treatment armamentarium. The current survey from the ALWP of EBMT aimed to assess the outcome [OS, LFS, NRM, RI] of a cohort of 1041 Ph+ALL patients who received allo-HSCT in CR1 between 2000 and 2010. The primary endpoint of the study was to assess the impact of the use of TKIs prior allo-HSCT considering the time period before and after 2007 when TKIs were made available in most EBMT centers for the treatment of adult Ph+ALL according to the European label extension for TKIs in adult Ph+ALL.
552 patients received alloHSCT from an HLA-matched related donor while 489 patients received an HLA-matched unrelated graft. 869 patients underwent a MAC regimen, while 172 received a RIC regimen (Table 1).
With a median follow-up of 20 months (range, 1-132) after alloHSCT, in the whole cohort, the 2-years OS and LFS were 54±2% and 42±2%, respectively. In multivariate analysis, NRM was significantly influenced by age>37 y. in the MAC subgroup (P<0.0001, HR=1.90, 95%CI, 1.40-2.57). No significant predictive factors for NRM were found in the RIC subgroup. The year of alloHSCT (≥2007) was a strong factor predictive of an improved LFS (P=0.001, HR=0.75, 95%, 0.63-0.89), while age>42 y. was associated with a lower LFS (P=0.001, HR=1.34, 95%CI, 1.12-1.6).
In the MAC alloHSCT subgroup, TBI and year of transplant ≥2007 were associated with significantly improved LFS (P=0.01, HR=0.75, 95%CI, 0.59-0.94; and P=0.005, HR=0.76, 95%CI, 0.62-0.92, respectively), while age>37 y. was a negative predictive factor for LFS (P=0.001, HR=1.38, 95%CI, 1.14-1.68). In the RIC alloHSCT subgroup, no significantly predictive factors were found for LFS.
When considering RI, multivariate analysis showed that the year of transplant ≥2007 was also associated with decreased relapse in both the MAC and RIC subgroups (P=0.003, HR=0.67, 95%CI, 0.51-0.88 and P=0.007, HR=0.50, 95%CI, 0.31-0.83, respectively). In the MAC subgroups, the use of TBI and an HLA-matched unrelated graft were found to be factors associated with decreased RI (Table 2).
This large survey suggests that the introduction of TKIs after the year 2007 within European centers, has likely improved the outcome of adult Ph+ALL patients eligible for alloHSCT. Prospective evaluation are needed since further improvement would be expected in the next few years with the wider use of minimal residual disease assessment associated to TKI-based preemptive and/or maintenance strategies.

**[P441]**

Table1-Patient characteristic

| | |
|---|---|
| **Median age** | **41,7 (18-73)** |
| **Gender of the patient (M/F)** | 600 (58%) /441 (42%) |
| **Interval from diagnosis to transplant** | 162 days |
| **interval from CR1 to transplant162** | 103 days |
| **Stem-cell source: BM/ PBSC** | 270 (29%) / 741 (71%) |
| **Donor type: HLA id. sibl/ MUD (6/6)** | 552 (53%) / 489 (47%) |
| **Conditioning: MAC/ RIC** | 869 (83%) / 172 (17%) |
| **TBI**<br>**- In MAC regimen**<br>**- In RIC regimen** | **766 (74%)**<br>719 (83%)<br>47 (27%) |

**[P441]**

Table 2- multivariate analysis for Relapse Incidence in MAC regimen

| RI in MAC | p value | HR |
|---|---|---|
| MUD vs HLA id | 0,03 | 0,75 |
| TBI | 0,008 | 0,66 |
| age>37y | 0,61 | 1,07 |
| year >=2007 | 0,003 | 0,67 |

**P442**

**Nelarabine as salvage therapy for highly resistant T-cell malignancies before allogeneic stem cell transplantation – a curative option**

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Background: Patients with T-cell acute lymphoblastic leukemia/ lymphoma (T-ALL/T-LBL) and failure to standard induction therapy or relapse have a poor outcome. The nucleosid analog nelarabine accumulates preferentially in T-lymphoblastic cells and has cytotoxic activity. Here we report on our experience with nelarabine in patients with relapsed T-ALL/T-LBL as induction therapy before allogeneic hematopoietic stem cell transplantation (alloHSCT).
Patients and Methods: Five patients were treated with nelarabine before subsequent alloHSCT at our institution. The median age was 32 years (range 24-41 years). Three patients suffered from relapsed T-LBL, one patient from failure of induction chemotherapy for T-ALL and one patient from relapse of T-ALL. Nelarabine was administered at a dose of 1.5 mg/m² on alternating days (day 1, 3, 5). One patient received only one cycle, two cycles were given in 4 patients. AlloHSCT was performed after conditioning with 12 Gy total body irradiation in combination with VP16 (60 mg/kg bodyweight, day -3) in 3 patients or cyclophosphamide (60 mg/kg bodyweight, day -3 and -2) in one patient. One patient received treosulfan (10 mg/m², day -6 to -2) and fludarabine (30 mg/m², day -6 to -2) as conditioning therapy before her second alloHSCT.
Results: The infusion of nelarabine was mostly well tolerated. Mild neurotoxicity occurred in two patients. All patients achieved at least partial remission of disease. Two patients died on day 63 or 137 after initiation of nelarabine treatment (day 21 and day 102 after alloHSCT) from severe sepsis. However, two patients (one with T-LBL and relapse after first alloHSCT and one without response to induction chemotherapy for T-ALL) are in continuous complete remission two and three years (day 1032 and 1337) after alloHSCT respectively. One patient developed mixed chimerism on day 302 after alloHSCT which was successfully treated with three courses of donor lymphocyte infusion. On day 540 he is in complete remission of T-LBL without any signs of relapse.
Conclusion: The T-cell directed drug nelarabine offers a curative option for the treatment of highly resistant T-cell malignancies in combination with subsequent alloHSCT and was well tolerated in this heavily pretreated cohort of patients.

**P443**

**Nelarabine-based salvage in adult patients with T-cell acute lymphoblastic leukaemia or lymphoblastic lymphoma relapsing after allogeneic stem cell transplantation: a French eperience**

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Introduction: Recently, prognosis of T-cell Acute Lymphoblastic Leukemia and Lymphoblastic Lymphoma (T-ALL/LL) has been re-assessed, based on minimal residual disease (MRD) levels and NOTCH1 pathway mutations. Allogeneic hematopoietic stem cell transplant (HSCT) in first complete remission (CR) remains the standard option in patients with a high risk of relapse. Patients relapsing after HSCT represent a very difficult challenge to get a second CR. Nelarabine has been associated with a high response rate in relapsing ALL, but few data are available on its efficacy and safety in the post-transplant setting. Patients. Medical records of 11 T-ALL/LL patients who received nelarabine-based salvage for a relapse after HSCT were retrospectively reviewed. These patients were treated with Nelarabine alone (1,5 g/m²/day (D) D1, D3, D5, every 28 days) (N=5) or nelarabine associated with hyperfractionated cyclophosphamide (HyperC; N=6). Results. Ten patients had T-ALL and one had T-LL. Median age was 23 years (14-62) at time of diagnosis. Ten patients underwent HSCT in first CR. HSCT conditioning regimen was myeloablative (N=7) or reduced (N=4). Source of stem cells was peripheral hematopoietic blood stem cells in 6 patients, bone marrow in 4 patients and unrelated cord blood in one patient. Transplant was related for four patients and unrelated for 7. Graft versus Host Disease (GVHD) prophylaxis consisted in ciclosporine alone (N=1) or associated with methotrexate (N=8) or mycophenolate (N=2). Eight patients presented grade I-II acute GVHD, no patient had grade III-IV. Two patients developed chronic GVHD (1 extensive). Relapse occurred with a median duration of 199 days (119-2099). Of the 11 patients treated with nelarabine-based salvage, 81% achieved hematological CR within a median delay of 48 days. At one year, disease-free and overall survivals were 70% and 90%, respectively. Eight patients received additional Nelarabine consolidation cycles (median, 4 cycles). Main toxicity was neurological, with 2 patients presenting sensitive neuropathy and cerebellar ataxia. Conclusion. In patients with T-ALL/LL relapsing after HSCT, nelarabine-based salvage is associated with very high 81% response rate, offering some

prolonged remissions are observed. Even if neurological toxicity might be an issue, this salvage is well tolerated. Post-transplant nelarabine maintenance might be a valuable option to investigate in high-risk patients, eventually driven by MRD detection.

**P444**

**Evaluation of plasma levels of adhesion molecules by biochip array technology in acute myeloid leukaemia patients**

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Objectives: Cytokines and adhesion molecules have been studied as markers of immune system activation in various diseases including hematological malignancies. The objective of our study was to evaluate plasma levels of adhesion molecules by biochip array technology in patients treated for acute myeloid leukemia (AML).
Methods: A total of 15 AML patients (mean age 48.7 ± 12.1 years, median 51, 8 males and 7 females) treated with cyclic chemotherapy (3+7, 2+5, HiDAC) alone or in combination with high-dose chemotherapy (preparative regimen Bu/Cy2 or Cy/TBI) followed by autologous hematopoietic stem cell transplantation were studied. We evaluated plasma levels of the following adhesion molecules: E-Selectin, L-Selectin, P-Selectin, Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1). All biomarkers were measured by biochip array technology on Evidence Investigator analyzer (Randox) at the diagnosis of AML (active leukemia) and at 6 months after completion of chemotherapy (durable complete remission/CR/ in all patients).
Results: Comparing plasma adhesion molecule levels in active leukemia and in durable CR, we found significant decrease in plasma E-Selectin (30.19 ± 20.46 mcg/L *vs.* 12.99 ± 8.00 mcg/L; p<0.01), L-Selectin (2179.35 ± 1169.39 mcg/L *vs.* 1533.35 ± 540.69 mcg/L; p<0.05), ICAM-1 (659.61 ± 259.50 mcg/L *vs.* 492.81 ± 236.96 mcg/L; p<0.05), VCAM-1 (716.22 ± 364.38 mcg/L *vs.* 514.52 ± 115.66 mcg/L; p<0.05). Plasma levels of P-Selectin were without significant difference (89.56 ± 67.60 mcg/L *vs.* 106.43 ± 52.74 mcg/L; ns).
Conclusion: Our results indicate that plasma levels of some adhesion molecules (E-Selectin, L-Selectin, ICAM-1, VCAM-1) are altered in patients treated for AML, showing activity of the disease. Whether these alterations could serve as a prognostic marker for AML is not known. Further studies in a larger number of patients and comparing adhesion molecule levels with established prognostic markers (cytogenetics, molecular genetics) will be needed to define the potential role of these and additional markers in the risk stratification of AML patients.
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**P445**

**No impact of HLA-C mismatch in patients with AML undergoing allogeneic haematopoietic stem cell transplantation**

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Background: Patients with acute leukemia undergoing allogeneic hematopoietic stem cell transplantation (HSCT) with unrelated HLA-C mismatched grafts have been shown to have worse outcome than patients receiving 8/8 matched unrelated grafts.
Aim: To evaluate the importance of HLA-C mismatch (MM) in patients with AML undergoing HSCT with unrelated donors between 2000 and 2010 in our center.
Patients and Methods: We retrospectively analyzed 137 patients, both adults (n=111) and children (n=26) with AML. Median age was 44 years (1-68). Forty-two patients received an HLA-C MM graft, 33 with one HLA-C antigen MM and 9 with an allele MM. All patients received antithymocyte globulin (ATG). Most of the patients had intermediate- (n=80) or adverse risk (n=22) AML. The majority of patients were transplanted in CR1 (n=73). Most patients (n=87) received a myeloablative conditioning regimen (MAC). Peripheral blood stem cell (PBSC) grafts were given to 108 patients and the vast majority (n=122) received cyclosporine A and metothrexate as graft-versus-host (GvHD) prophylaxis.
Results: Overall 5-year survival for the 137 patients was 50%, relapse-free survival (RFS) was 46% and TRM at 100 days and 1 year post HSCT was 13.5% and 20.3%, respectively. Patients with HLA-C MM had a 5-year overall survival of 54% compared to 51% in those with HLA-C match (ns). RFS in patients with HLA-C MM was 52% as opposed to 45 % in patients with HLA-C match (ns). Furthermore both groups had a TRM 1 year post HSCT of 17% (ns) and acute GvHD grade 2-4 was diagnosed in 24% of patients with HLA-C MM and 25% in those with HLA-C match (ns). Also the incidence of chronic GvHD was similar with 23% in patients with HLA-C MM and 22% in patients who received HLA-C matched grafts (ns).
In the multivariate analysis more advanced AML was associated with transplant failure (HR=2.02; p<0.01) and relapse (HR=2.99; p=0.001). Furthermore, the most important risk factors for aGvHD and cGVHD were reduced intensity conditioning (HR=6,36; p=0.002) and PBSC grafts (HR=6,05; p=0.004), respectively. Severe acute GvHD (grades III-IV) increased the risk of TRM (HR=10.2; p<0.001) and was also the most influential risk factor on mortality (HR=5.35; p<0.001) but in none of these analyses was HLA-C MM shown to be a significant risk factor.
Conclusion: In the present study HLA-C MM had no effect on outcome which may be explained by the use of ATG. Therefore, unrelated donors with HLA-C MM could be acceptable for patients with AML.

**P446**

**Novel risk factors in acute myeloid leukaemia as predictors of short failure free survival can be overcome by stem cell transplantation**

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Introduction: We have recently reported that CD25 positive AML is associated with high relapse rate and short failure free survival (FFS). CD25+ was a more robust predictor of AML relapse (multivariate Cox regression analysis HR 6.54 [1.34-9.15], p=0.01; FLT3-ITD: HR 4.72 [2.04-10.92], p= 0.03; Cerny *et al* Blood 2011; 118: abstr. 3560). Similarly FLT3-ITD has been associated with poor outcome in AML patients (pts). We report the effect of stem cell transplantation (SCT) on outcome of pts with CD25+ or FLT3-ITD+ AML.
Methods and Patients: We have retrospectively evaluated 46 AML pts eligible for induction chemotherapy (APL was excluded). Median age was 60 years (29-83), 15 (33%) pts were older than 65; 21 (47%) females. The expression of CD25 or presence of FLT3-ITD was assigned as high risk AML (HR-AML) and outcome was compared with the rest of pts. No statistical difference in distribution of the following characteristics: sex, age (65+), cytogenetic risk, SCT between high risk and other pts was seen. 67% of pts with CD25+ AML were FLT3-ITD+ (p=0.005); NPM1mut clustered with FLT3-ITD (p=0.036). As induction high dose cytarabine/anthracycline based regimen was used in 40 (89%) pts, and 5 (11%) pts received 7+3. 23 (51%) pts received stem cell transplantation (SCT). Median time from diagnosis to SCT was 4 months (2-7).
Results: The estimated median FFS for pts with both (CD25+ and FLT3-ITD+) risk factors was 1.9 months, with one risk factor (CD25+ or FLT3-ITD+) was 10.8 months and not reached in

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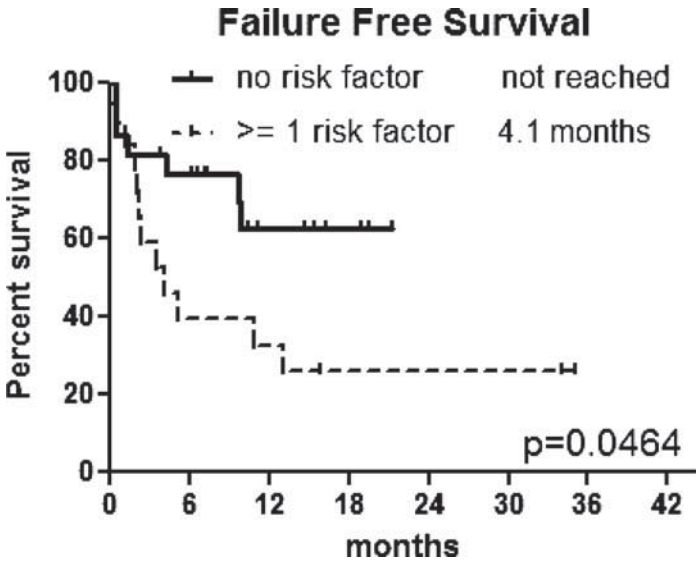


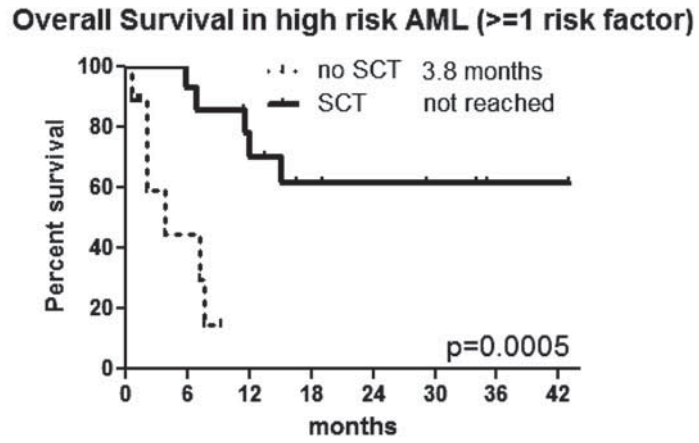


pts without these risk factors (CD25- and FLT3-ITD-; p=0.0016). This did not translate into a difference in OS among these groups (1-year OS: 46% *vs* 54% *vs* 70%, p=0.63). Pts undergoing SCT had significantly longer 1-year OS (75%) compared to pts without SCT (35%; p=0.0019). In multivariate analysis SCT was a predictor for improved OS (HR 0.22, p=0.004). Pts with HR- AML who received SCT had also significantly longer 1-year OS (70%) compared to pts who did not receive SCT (10%; p=0.0005). At the time of SCT 8 (67%) HR-AML pts were in CR1 and 4 (33%) were in CR2 or had refractory disease. Out of the remaining pts 12 (92%) were in CR1 and 1 (8%) pt had relapse at the time of SCT. Conclusion: The expression of CD25 and/or presence of FLT-ITD characterize poor prognostic subgroup in AML with a short FFS and high risk of relapse. In our experience treatment with SCT appeared to abolish the negative impact of CD25+ and FLT3-ITD+ on OS.

**P447**

**Normal karyotype AML has a similar outcome post haematopoietic stem cell transplantation irrespective of FLT 3 ITD mutation status at diagnosis**

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Acute myeloid leukaemia (AML) with an internal tandem duplication of the FMS like tyrosine kinase receptor mutation (FLT pos-ITD) has a poor prognosis. The data as to whether allogeneic haematopoietic stem cell transplantation (HSCT) in this setting overcomes the poor prognosis is limited. The purpose of this study was to review the outcome of normal karyotype (NK) AML in patients with or without FLT 3 ITD mutation following HSCT.
We have performed 62 HSCTs in patients with intermediate risk AML from 1 January 2006 to 1 December 2011. Of this cohort, 46 (74%) (Male=24) had NK AML. Patient characteristics are outlined in Table 1. The median age was 47yrs (range 20-60 yrs). The donor type was related in 67% (n=31) and the donor source was BM in 16 (35%) and PBSC in the 30 (65%). 22 received myeloablative and 24 received reduced intensity conditioning. 30 (65%) were in first complete remission (CR1) at the time of transplant and the remaining were beyond CR1. 21 were positive for FLT 3-ITD mutation at diagnosis. The median follow up from date of transplant was 536 days (range 32-1959).
Patients who were NK FLT3 ITD positive had a similar outcome post transplant to those who were NK FLT3 ITD negative at diagnosis. This is shown in Figure 1. Donor type, donor source and conditioning regimen did not have a significant impact on outcome. 34 (74%) patients are alive at date of last follow-up and relapse post transplant had a dismal prognosis irrespective of FLT3 ITD status.
In conclusion, patients with NK FLT3 ITD mutation positive AML had a comparable overall survival to those patients with FLT3 ITD mutation negative AML. This data supports the use of allogeneic transplantation in CR1 in all patients with FLT-3 ITD mutation positive AML who have a suitable donor. Further studies in this patient population are necessary to define the relationship of additional molecular markers and the outcome following HSCT.

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## Table 1. Patient Characteristics

| | n=46 |
|---|---|
| Age (y), median (range) | 47(20-64) |
| Sex (male/female) | 24/22 |
| CR1/>CR1 | 30/16 |
| FLT3-ITD mutation (pos/neg) | 21/25 |
| Donor | |
| •Related/Unrelated | 31/15 |
| •BM/PBSC | 16/30 |
| Conditioning | |
| •MA/RIC | 22/24 |
| Median follow up, days (range) | 536, (32-1959) |

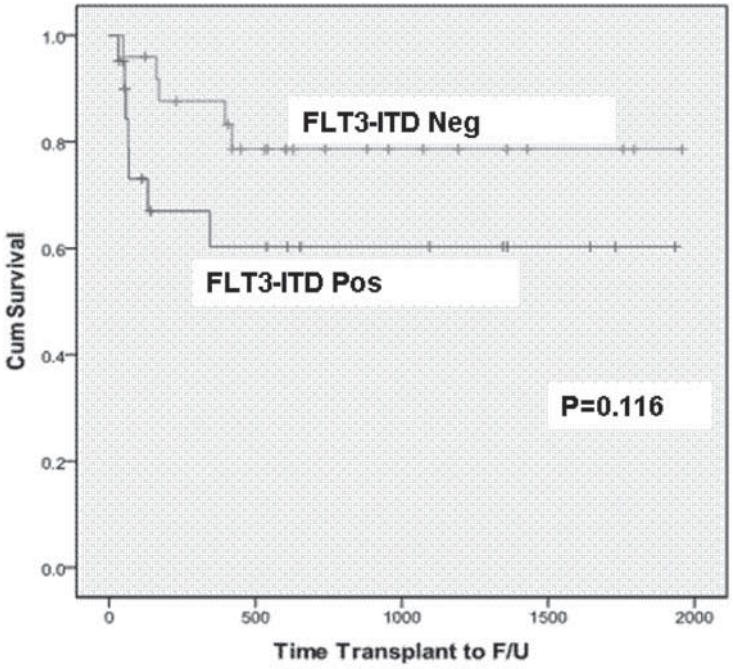


**Figure 1. Overall survival in normal karyotype AML**

**P448**

**Prognostic relevance of WT1 molecular levels in patients with acute myeloid leukaemia after allogeneic stem cell transplantation**

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Introduction: WT1 is a panleukemic marker that is highly expressed in the majority of Acute Myeloid Leukemias (AML). Monitoring WT1 levels at different time points is very useful to assess disease status. The present study aimed to investigate the correlation between WT1 levels and clinical outcome in patients with AML after Allogeneic Stem Cell Transplantation (Allo-SCT).
Patients and Methods: From January 2010 to present, 11 patients with AML who underwent to Allo-SCT in our Transplantation Unit, monitored bone marrow WT1 molecular levels before and every 3 months after transplantation. All the samples have been analysed with Real Time PCR as described by Cilloni *et al* (JCO, 2009). Levels of WT1 are expressed as WT1 copies/ABL copies x $10^4$. Normal range in bone marrow is <250 WT1 copies/ABL copies x $10^4$.

Results: At diagnosis all patients expressed high levels of WT1 with a median value of 11.184 WT1 copies/ABL copies x $10^4$ (range 1.325-18.338). After induction therapy the median fell to 655 (range 12-5.868) and at pre-transplantation time to 26 (range 7-986). Four out 11 patients (36%) relapsed 1, 3 and 6 months after bone marrow transplantation. At pre-transplantation time the median value of relapsed patients was higher than the median value of patients who maintained complete remission (182; range 20-986 *vs* 15; range 4-65).
Conclusion: The assessment of WT1 levels at pre-transplantation time could be an important marker to predict the risk of relapse in patients with AML.

**P449**

**Influence of natural killer cells alloreactivity in outcome to allogeneic bone marrow transplant based to genotyping KIR/HLA**

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The allogeneic hematopoietic stem cell is the most appropriate therapy for adults leukemias. Studies show that lack of a single ligand HLA to patient for donor KIR ligand can trigger NK cytotoxicity by the absence of inhibitory signals and cause graft versus tumor effects and increase overall survival. The objective of this study was to evaluate the NK alloreativity based on the presence of KIR/HLA ligands between donors and patients who underwent allogeneic bone marrow transplant. From March 2009 to April 2011, 31 patients and donors were analyzed for NK alloreativity. Genomic DNA of donors and recipients were extracted using the EZ-DNA kit. The samples were typed by PCR-SSO HLA-A, B, C and DR and DQ 14 KIR genes and 2 pseudogenes. The amplified product was hybridized with specific probes attached to microspheres for HLA and KIR genes and alleles. The readings of the reactions were performed in flow cytometry using Luminex technology and organized by the computer program Labscan ®. The samples were analyzed (HLA program FusionTM Research, One Lambda) for the presence and absence of KIR and HLA genes. Statistical analysis was conducted using the SPSS and the significance level was 5%. To investigate a possible influence of polymorphisms of these genes in the occurrence of post-transplant was performed a univariate Cox survival curve with the Kaplan-Meier probability curve. Analyzing the frequency of diseases was observed that acute myeloid leukemia was the most occurred (35.5%), followed by chronic myeloid leukemia (19.4%), malignant lymphomas and lymphoid leukemia. Of the 31 patients, 18 (58%) showed lack of HLA-C ligand for KIR receptor donor, while 13 patients (42%) showed all the ligands presents. Overall survival analysis showed in the group that lack the ligand HLA-C for KIR presented 88% survival, suggesting occurrence of post-transplant aloreativity NK and possible increase in graft-versus-tumor effect, whereas the group presented all the ligands the survival rate was 31% ($p = 0.03$). Initial results show a protective effect and survival in the absence of ligand HLA-C in patient of the KIR inhibitory donor, showing a possible aloreativity. Further tests will be conducted to establish the best correlation between KIR and HLA to donor and recipient in allogeneic bone marrow transplantation.

**P450**

**Quantitative expression of Toll-like receptor -1, -2, -3, -5, -7, and -9 in blasts of patients with newly diagnosed or relapsed acute myeloid leukaemia**

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Objectives: Toll-like receptors (TLRs) are well known to play an essential role in the immune response. As previously described TLR-2, -4, and -9 are expressed on different levels in dendritic cells derived from blasts of patients with acute myeloid leukemia (AML). It is known that dendritic cells express TLRs which identify so called pathogen-associated molecular patterns (PAMPs) and by this way trigger the maturation of dendritic cells and production of cytokines. By this way the innate and adaptive immunity are activated. The aim of our study was to evaluate the expression of TLR-1, -2, -3, -5, -7, and -9 in blasts of patients with AML and compared it the expression of mononuclear cells of healthy volunteers.
Methods: We analysed the expression of the above mentioned TLRs with a quantitative real-time polymerase-chain-reaction in blasts generated of whole blood of 6 patients with newly diagnosed or relapsed AML, the AML cell-line Kasumi 1, and mononuclear cells of healthy volunteers. ABL was used as endogenous reference. The median patients age was 57 years (range 38-60 years). Three patients had relapsed AML after allogeneic stem cell transplantation (aSCT), 2 patients a relapse before aSCT, and 1 patient had a newly diagnosed AML. AML classification was as follows: M4 n=2, M5 n=2, unspecified n=2.
Results: In AML-blasts and in mononuclear cells of healthy volunteers all analysed TLRs were highly expressed. TLR-1 and -2 were seen to be expressed at a lower level than compared to TLR-3, - 5, - 8- and -9, respectively. The highest level of TLR expression was seen for TLR-7. We could see a slightly different expression of TLR-2 and -3 in healthy volunteers compared to patients with AML. For TLR-2 we had a higher expression in healthy volunteers with a median range of 9936% compared to 1888%. For TLR-3 the level for healthy volunteers was lower in contrast to the level of patients with AML (median 156862% and 487949%, respectively). But due to the small numbers of analyses this difference was not significant.
Conclusion: We could demonstrate in our study an expression of all tested TLRs in leukemic blasts. There was no significant difference compared to the expression of TLRs in healthy volunteers. We could detect a slight difference for TLR-2 and -3, but due to the small number of patients, further analyses are needed to confirm these results.

**P451**

**The presence of minimal residual disease monitored by quantitative assessment of WT1 gene before allogeneic stem cell transplantation in patients in first remission of acute myeloid leukaemia has an impact on their future prognosis**

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Purpose: The high risk AML patients may benefit from the allogeneic stem cell transplantation (allo-SCT) as a consolidation of first complete remission (CR1). In the absence of an universal marker for minimal residual disease (MRD), little information is still about the importance of MRD prior to allo-SCT. The aim was to retrospectively evaluate the significance of WT1 status in AML patients treated with allo-SCT in CR1.
Patients and Methods: Overall 42 patients (pts) in hematological CR1 were transplanted from April 2005-July 2011. Median age was 47 years (range; 20-63), 18 men, three good risk, intermediate risk 23, high risk 11 (NA 5). A total of 21 pts achieved CR1 after salvage therapy,12 pts were in iCR1. In 33 pts were used PBPC, in 9 pts bone marrow. The donors were identical

siblings in 15 pts, (2x mismatched siblings), matched unrelated donors in 15 pts and mismatched UDs in 10 pts. Conditioning was myeloablative in 34 pts, RIC in 8 pts. Median follow-up was 15 months (range; 2-77). The expression of WT1 gene was measured by real-time polymerase chain reaction in peripheral blood according the European Leukemia Net recommendations. At the time of allo-SCT 29 pts were WT1 negative and 13 pts were WT1 positive.
Results: When comparing the two groups according the WT1 status, there was not significant difference in therms of the age,leukocyte count at dg, sex, AML type, genetic risk, primary induction failure, number of iCR, induction or consolidation type. Also, donor type, graft type, conditioning regimen, HSCT-CI, aGVHD or cGVHD incidence were not significantly different.
In the univariate analysis, in terms of OS, there was a trend toward better survival in the group of WT1 neg (OS in 3 years 77% *vs* 56%, p=0.06), in terms of PFS, significantly better results were observed in group of WT1 neg (PFS in 3y 79% *vs* 32%, p=0.001) and in pts without aGVHD (p=0.001). In multivariate analysis, the only significant in terms of better OS was WT1 negativity (p=0.029), in terms of PFS, again WT1 negativity (p=0.015) and the absence of aGVHD (p=0.011).
Conclusion: Our results show that MRD status measured by quantitative assessment of WT1 gene in AML patients in CR1 significantly affects their future prognosis after allo-SCT. WT1 positive patients should be considered for more intensive pretransplantation therapy or earlier immunomodulatory intervention after allo-SCT (pre-emptive DLI). The larger prospective studies are necessary to confirm this hypothesis.

**P452**

**The prognostic value of cytogenetics in influencing the outcome of acute myeloid leukaemia after allogeneic stem cell transplantation**

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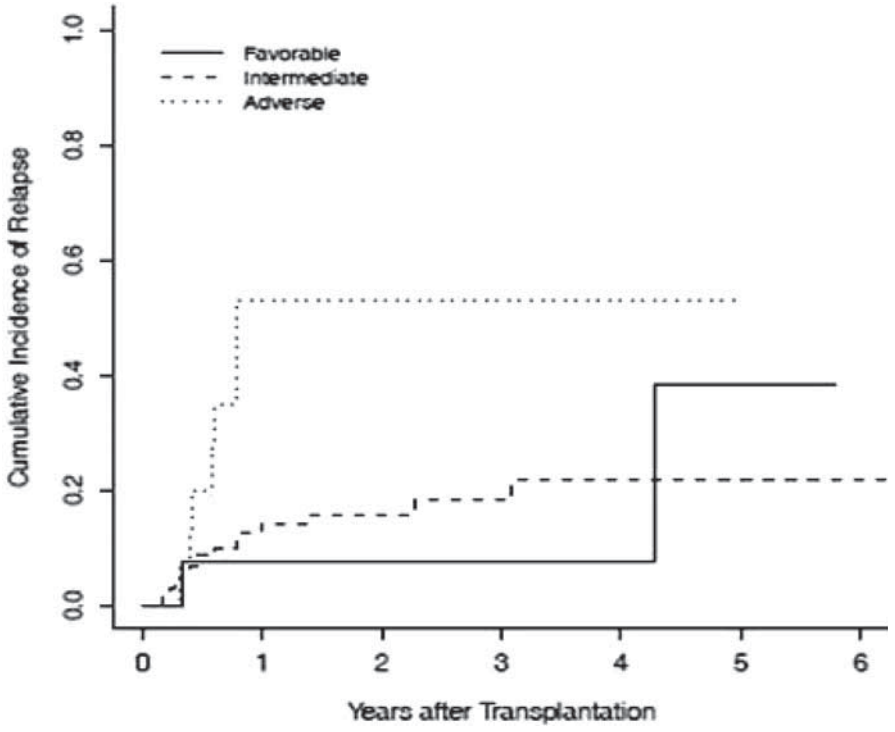
Introduction: Diagnostic karyotype is the most significant prognostic factor in AML patients and provides the framework for current risk-stratified treatment approaches. The aim of our study was to use this score for outcome prediction after hematopoietic stem cell transplant (HSCT) in first complete remission.
Methods: We studied 171 AML patients (53.8% male) with a median age of 26 years (range: 18-38 yrs) who underwent HSCT in first CR at Hematology, Oncology and Stem cell transplantation Research center. The median follow-up was 1 year (1-82 months). We analyzed the diagnostic karyotypes and grouped patients according to the Medical Research Council/National Cancer Research (MRC) Institute trials and Southwest Oncology Group (SWOG) scheme. We compared the 3 existing cytogenetic groups of favorable (8% in MRC and 5% in SWOG), intermediate (82% in MRC and 85% in SWOG) and adverse (10% in each group) to stratify patients for OS and LFS after HSCT.
Result: The hazard of death in favorable group of MRC cytogenetic risk categorization was significantly lower than adverse group (HR=0.1; 95% CI: (0.01, 0.79); P=0.03), as well as SWOG grouping system (HR=0.08; 95% CI: (0.001, 0.69); P=0.02). It was also significantly lower in intermediate group compared to adverse group (HR=0.31; 95% CI: (0.14, 0.70); P=0.005) in MRC and SWOG (HR=0.37; 95% CI: (0.17, 0.91); P=0.03). The hazard of relapse or death in favorable group of MRC was significantly lower than adverse group (HR=0.14; 95% CI: (0.03, 0.64); P=0.01), as well as SWOG (HR=0.11; 95% CI: (0.01, 0.84); P=0.03). It was also significantly lower in intermediate group of MRC than adverse group (HR=0.3; 95% CI: (0.15, 0.62); P=0.001) and SWOG (HR=0.32; 95% CI: (0.16, 0.65); P=0.002). Cumulative incidence of relapse (95% CI) one year after HSCT in favorable group was 8% (0.004-0.3), intermediate group 14% (0.08-0.22) and adverse group 53% (0.21-0.77) (P=0.01) (Figure 1).
Conclusion: The results obtained from MRC and SWOG groups were similar and in consistent with those of previous studies. By analyzing the larger cohort and more follow-up, karyotype as a critical independent determinant of outcome and also as a means of optimizing therapy would help in risk stratification of AML. It seemed that patients in favorable group had a longer DFS after HSCT. No significantly longer DFS was found to be present in unfavorable group even after HSCT but only a trend to a better prognosis. The results of the study revealed that the prognosis of intermediate group improved after HSCT.

**[P452]**



**P453**

**Pre-transplant HLA mistyping in diagnostic samples of acute myeloid leukaemia patients due to acquired uniparental disomy**

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Genome wide single nucleotide polymorphisms array analyses have shown that acquired uniparental disomy (aUPD) by mitotic recombination was not uncommon in AML. Although aUPD involving chromosome 6 has been recently documented in relapse AML, pretransplant aUPD involving the HLA region is poorly documented. Such events could be of great interest because loss of heterozygosity (LOH) resulting from aUPD in leukemic cells may lead to incorrect results if HLA typing for hematopoietic stem cell (HSC) donor searches is performed on blood samples drawn during blastic crisis.

We report here six AML patients whose HLA typing was performed on DNA extracted from peripheral blood lymphocytes (PBLs) obtained at diagnosis. By using PCR-SSO hybridization on microbeads (luminex technology) and bi-allelic sequence-based typing, apparent LOH was observed in all cases. In 3 patients LOH was detected for the entire HLA region, whereas HLA-A,B,C LOH only was detected in 2 patients and HLA-A LOH in 1 patient. DNA extracted from the PBLs at diagnosis (85-95% blasts) was labeled and tested by Comparative Genomic Hybridization on microarrays (aCGH). The results showed that the copy number was neutral for the HLA-A,B,C,DRB1,DQB1 and DPB1 loci. Because no chromosomal loss has been observed, the LOH observed in all 6 patients can be explained by acquired UPD involving 6p21. When performed on PBLs obtained after complete remission of AML, HLA typing of the patients lead to the identification of both haplotypes. In order to determine a detection threshold of the PCR-SSO reverse hybridization on the microbeads assay, an increasingly used test for routine HLA typing, we performed DNA mixing experiments of 2 HLA homozygous samples. The threshold for detection of the second HLA-A,B,DRB1 allele was >10% when present at a double dose.

We conclude that PCR-based HLA typing techniques using locus-specific primers could miss the second haplotype if haplotype loss has occurred in >80% of the nucleated cells in the blood sample, as observed during acute blastic crisis. Because aUPD may be partial any homozygous HLA result should be confirmed by a second typing performed either on a buccal swab or on a blood sample drawn from the patient in remission.

**P454**

**Extramedullary relapse after allogeneic bone marrow transplantation and transfusion of donor lymphocytes**

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Introduction: Chloroma, also known as granulocytic sarcoma (GS), is an inclusion of early myeloid stem cells in different tissues in patients with leukaemia. Isolated extramedullary relapses of GS after allogeneic bone marrow transplantation (BMT) are reported as an unusual event.

Materials and Methods: We analysed 6 patients suffering from acute lymphoblastic (ALL, n=2) or myeloid leukaemia (AML, n=4) who developed a GS after BMT. The data of relapse are based on histological, cytological and molecular investigations.

Results: All patients (pts) had a history of advanced (n=3) or refractory disease (n= 3) before BMT and received transplants from matched related (n= 5) or unrelated donors (n=1). In one patient GS was suspected with initial diagnosis and regressed under induction chemotherapy. Cyclosporin A and short MTX were used as post transplant GvHD prophylaxis in all patients. GS developed simultaneously (2/6 pts) with bone marrow relapse or after graft versus leukaemia therapy either by transfusion of donor lymphocytes (DLI) (3/6 pts) or discontinuation of immunosuppression (1/6 pts) (Table 1). In the latter patients, the interval from bone marrow relapse to extramedullary relapse was in the range of 2 to 17 month. In two of these four patients, GS developed without new bone marrow involvement. GS primarily developed in subcutaneous tissue (n=2), paravertebral (n=2), testis (n=1) or in pelvis (n=1). In the course of the disease GS was also found in other locations as well (glottis, spine, breast).

Conclusion: Patients receiving allogeneic transplantation for refractory or advanced disease may have an increased risk to develop GS. In addition the induction of a graft versus leukaemia effect may increase the risk for GS development and remission control in the bone marrow alone may not always be sufficient.

**[P454]**

| Patient | BMT | Stop immuno-suppression | Bone marrow relapse | Transfusion of DLI | Extramedullary relapse: localisation |
|---|---|---|---|---|---|
| **1 (ALL)** | 01/11 | 08/11 | 08/11 | - | 10/11: testis |
| **2 (ALL)** | 03/10 | 04/10 | 04/10 | 07/10<br>11/10<br>01-02/11 | 08/10: paravertebral<br>12/10: lumbar spine<br>04/11: progress in spine |
| **3 (AML)** | 02/10 | 05/10 | 05/10 | 07/10-08/10 | 10/11: subcutaneous tissue |
| **4 (AML)** | 12/10 | 05/11 | 11/11 | - | 11/11: paravertebral |
| **5 (AML)** | 03/05 | 07/08<br>(not stopped before because of limited cGvHD) | 07/08 | 12/08 01/09 | 07/08: pelvis<br>01-03/09: subcutaneous tissue |
| **6 (AML)** | 11/08 | 04/09 | 04/09 | 06/09-12/09 | 10/09: subcutaneous tissue<br>03/10: breast<br>10/10: glottis |

*Table 1: Data from patients*

**P455**

**Extramedullary relapses following allogeneic haematopoietic stem cell transplantation for acute myeloid leukaemia**

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is potentially curative therapy for patients with acute myeloid leukemia (AML), but relapses are one of the major causes of treatment failure. Extramedullary relapses (EMRs) in AML are long-term complications. The incidence of EMRs after allo-HSCT is rather low–in EBMT study published in 1996 it was reported as 0.65%. The aim of our retrospective study was to determine incidence, characteristics and outcome of EMRs in consecutive patients (pts) who underwent allo-HSCT for AML in seven Polish transplant centers between January 2000 and October 2011.

Patients and Methods: We evaluated incidence of EMR in-770 consecutive patients with median age-32 (range 8 months-65 years). 26 pts aged-32,2 (range 11 months–50 years) with EMR were identified. All pts (9-women, 17-men) were diagnosed with AML according to FAB classification: M0-1(3,8%), M1-4(15,5%), M2-5(19,2%), M3-1(3,8%), M4-10(38,4%), M5-4(15,5%), AML other-1(3,8%). Allo-HSCT from matched sibling donors (88,5%) and unrelated donors (11,5%) were performed after conditioning based on chemotherapy (no patient was conditioned with TBI-based regimen). 19 (73,1%) recipients were transplanted in the first complete remission (CR1), 5 (19,2%) in the CR2 and 2 (7,7%) > CR2 respectively. The EMRs were diagnosed in the median time of 12-months during post-transplant period (range 2-72). The most frequent relapse sites included: 1) central nervous system (9; 34,6%), 2) skin (5; 19,2%), 3) breast tissue (4; 15,5%). 21 pts (80,8%) were not receiving any immunosuppressant at the moment of EMR diagnosis. Treatment for EMR included: systemic chemotherapy in-23 (88,5%) pts, local radiotherapy in-11 (42,3%), donor lymphocyte infusions in-5 (19,2%), surgery in-3 (11,5%), second alloHSCT in-7 (26,9%) or combination of above-mentioned methods. Despite intensive therapy 18 (69,2%) patients died, median time of survival was 12 months (range 6 days–70 months).

Results: Incidence of EMRs in presented group was 3,38%. EMRs were more frequently related to AML M4/M5 – 14 pts (53,8%). Overall survival for this group was 30,8%.

Conclusions: EMRs are long-term serious complications following allo-HSCT for AML, with a very poor prognosis. There is no established efficacious treatment for this form of relapse. Our observations suggest that prospective studies are needed to define risk factors, methods of early detection and novel therapeutic options for these patients.

**P456**

**Higher frequency of relapses with granulocytic sarcoma after allogeneic haematopietic stem cell transplantation than with chemotherapy in adult acute myeloid leukaemia patients**

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Objectives: Granulocytic sarcoma (GS) are extra-medullary tumors composed of malignant granulocytic precursor cells. Several investigators reported that the high frequency of relapses with accompanying GS after allogeneic hematopoietic stem cell transplantation (allo-HSCT), however, the study has not been available, which evaluate the frequency compared between after allo-HSCT and chemotherapy. To fill this research need, we conducted this large-scale retrospective analysis which explored the accurate frequency and the risk factors for development of GS.

Methods: From January, 1990 to March, 2010, 498 consecutive adult patients (median age 57 (15-89), male/female; 301/197) diagnosed as AML were included, and 116 patients received allo-HSCT. The chi2-test was used for comparison of binary variables. The Mann-Whitney U test was used for comparison of continuous variables. Overall survival (OS) was estimated by the Kaplan-Meier method, and compared using the log-rank test. $P < 0.05$ was considered as statistically significance.

Results: A total of 245 relapses was occurred in 193 patients; 27 relapses (11.0%) were with GS and 36 relapses were occurred after allo-HSCT. Among 36 relapses after allo-HSCT, 9 (25%) occurred GS, and out of the 9 relapses 3 were developed as isolated GS. The frequency of relapses with GS after allo-HSCT was significantly higher than that after chemotherapy (25% *vs* 9%; p=0.008). Among 36 relapses after allo-HSCT, there was no significant difference between with and without GS in conditioning regimen (with or without total body irradiation and myeloablative or not) and stem cell source (related, unrelated, or cord blood), likewise, the presence and severity of both acute and chronic graft-versus-host disease did not affect the frequency of GS. Additionally the durations from transplant to relapse were also comparable between with and without GS. Among 33 relapses (8 with GS and 25 without GS) limited in the first allo-HSCT, the 1-year OS rates after transplant for patients with GS showed the tendency of favorable prognosis compared with those without GS (25% *vs.* 12%, respectively; p = 0.08).

Conclusion: Our findings indicated that the vigilance is required regarding the relapse with accompanying GS after allo-HSCT, which is received from any kind of donor source and conditioned with any regimen. Furthermore, detection of GS at relapse after allo-HSCT may enable us to identify the patients with favorable prognosis.

**P457**

**Efficacy and toxicity of non-T-cell depleted haploidentical stem cell transplantation in children with refractory or relapsed acute leukaemia**

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Background: Non-T-cell depleted (non-TCD) HLA-haploidentical SCT (haplo-SCT) is a form of adoptive cellular therapy that has a high degree of efficacy in hematologic malignancies. Previously we reported the safety profile assessing GVHD prophylaxis that was conducted with anti-human thymocyte immunoglobulin(ATG), tacrolimus, methotrexate(MTX) and prednisolone(PSL) in non-TCD haplo-SCT (Kikuta, Clin Transplant, 2010). We evaluated efficacy and toxicity of non-TCD haplo-SCT in children with very high risk refractory/relapsed acute leukemia (VHR-R/R AL).

Methods: VHR-R/R AL was defined by the 1-year survival rate less than 30%. From Aug 2000 to April 2011, consecutive 16 patients (pts) with VHR-R/R AL who underwent non-TCD-haplo-SCT were included. The median age of pts was 7.7(0.5-17.9) years old. The diagnosis included ALL (10), AML (3), M/NKL (3). The disease status at non-TCD-haplo-SCT were 4 in CR2 (MLL rearrangement: 1 pt, Ph positive: 1 pt, after SCT: 2 pts), 12 in non-CR (after SCT: 5 pts, after chemotherapy: 7 pts). HLA disparities were 3/8 in 1 pt, 4/8 in 15 pts. Donors included fathers (9), mothers (5), and siblings (2). Fifteen pts received myeloablative conditioning (TBI based: 11 pts, Bu based: 4 pts) and 12 pts of them received ATG (rabbit, thymoglobulin 2.5 mg/kg) containing regimen. The GVHD prophylaxis was conducted

with tacrolimus, MTX and PSL. Thirteen pts received peripheral blood stem cells and 3 pts received BM.
Results: All pts achieved engraftment (median 14 days for neutrophils). All pts achieved CR in non-remission at SCT. Acute GVHD grade 2-4 and grade 3-4 occurred in 12/16 pts (75%) and 2/16 (13%), respectively. Chronic GVHD occurred in 7/13 (54%). The treatment-related complications observed within day+100 included: viral reactivations (14 pts), Candida sepsis (2 pts), Aspergillus (1 pt), Bacterial sepsis (2 pts), hemorrhagic cystitis (2 pts), thrombotic microangiopathy (1 pt), and posterior reversible encephalopathy syndrome (1 pt). Non-relapsed mortality occurred in 3 pts and relapse occurred in 3 pts. With the median follow-up of 15 (1.5-134) months, 1-year and 2-year event free survival were 69% and 59%.
Conclusions: These data suggest that non-TCD haplo-SCT combined with our GVHD prophylaxis is well tolerated, facilitate engraftment, and has significant anti-tumor activity, particularly in pediatric patients with non-remission acute leukemia. The safety profile is acceptable in this refractory/ relapsed population.

**P458**

**Cytogenetic and molecular characteristics of post-transplant relapses in patients with acute leukaemias**

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Introduction. Post-transplant relapses (PTR) are occured in about third of patients (pts) with acute leukemias (AL) independently of the type of transplantation.
Aim. Since cytogenetic and molecular characteristics of PTR are still obscure, 80 such pts were analyzed by means of standard G-banding and partly by M-FISH. Furthermore, expression levels of genes WT1 and EVI-1 were determined by real-time PCR analysis in half of them.
Results. The main our cytogenetic finding was often complex chromosome abnormalities (CCA) which were presented in over 40% of cases with PTR including those with Ph+ acute lymphoblastic leukemia (ALL). Besides, abnormalities of chromosome numbers 5 and 7 (-5/5q- and -7/7q-) as well as trisomy 22 were among non-seldom in the pts with AML PTR whereas it was trisomy 22 in pts with Ph+ ALL PTR. As for interesting molecular findings, they include an increased expression of WT1 gene in 69% of pts with AML PTR. Furthermore, the increased expression level of EVI-1 gene was registered in 5 pts, including 1 with t(2;3)(p11;q26) and CCA, 2 pts with t(3;21)(q26;q22) complicated with t(8;9)(p11;q34) or trisomy 13, and 2 pts with CCA without chromosome 3 aberrations. According to published data these cytogenetic and molecular changes are strongly associated with a great resistance to both chemotherapy and hematopoietic stem cell transplantation (HSCT), although they were treated effectively by means of low doses of such hypomethylating agents as Decitabine (Janssen) or Vidaza (Celgene). Among other chromosome abnormalities in pts with PTR must be mentioned non-random rearrangements of 11q23 locus with gene MLL which took place in both AML and ALL leukemias.
Conclusion. Since cytogenetic characteristics of PTR are very informative they are needed the further investigations in both theoretical and practical aspects.

**P459**

**Immunoediting of the genomic and transcriptional profile of acute myeloid leukaemia in response to allogeneic haematopoietic stem cell transplantation**

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Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) can grant long-term cure of Acute Myeloid Leukemia (AML) thanks to the antitumor effect of the transplanted immune system. Still, relapse remains an issue: in the haploidentical setting, we showed in a landmark study that disease recurrence can be frequently explained by de novo genomic loss of the mismatched HLA in leukemia, leading to specific immune evasion (Vago, NEJM, 2009).
In this study, we set out to identify new mechanisms of leukemia immune escape and relapse in different HSCT settings, by performing comparative high-throughput genomic and transcriptional profiling of AML blasts harvested at diagnosis and relapse after allogeneic HSCT.
Whole genome Single Nucleotide Polymorphism profiling demonstrated de novo macroalterations in 6/11 relapses studied to date. Uniparental disomy was the most frequent event, often involving the FLT3 locus. In 5/11 cases we observed clonal redistribution at relapse, with relative expansion of clones displaying macroalterations. Importantly, we documented frequent positive selection for adverse prognostic mutations: in particular, in 5/6 patients carrying the FLT3 Internal Tandem Duplication (ITD), the allelic burden of the mutated form was increased at relapse.
Gene expression profiling was performed for 7 paired diagnosis-relapse samples. Interestingly, in the 3 patients with the earliest timing of disease recurrence (median time after HSCT 30 days), most of the transcripts deregulated at relapse were part of immune processes. Moreover, in one of these patients, who relapsed early after a haploidentical HSCT, we could document specific down-regulation of the HLA class II antigen presentation pathway, and its recovery when leukemic cells collected at relapse were transferred into immuno-compromised NOD/SCID mice, suggesting plasticity of the deregulation based on the degree of immune pressure.
In conclusion, we demonstrate that genomic and transcriptional alterations occur frequently at leukemia relapse after allogeneic HSCT. The preferential outgrowth of leukemic cells displaying specific unfavorable mutations (such as FLT3-ITD) grants a biological rationale for the post-transplantation use of targeted inhibitors. Moreover, the observation that not only genomic but also transcriptional HLA loss can be at the basis of relapse after haploidentical HSCT warrants further optimization of monitoring and molecular re-typing for targeted treatment of disease relapse.

**P460**

**Short-hairpin RNA interference with CD44v6 reveals a complex role in chemoresistance and leukemogenicity**

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Acute myeloid leukemia (AML) is highly sensitive to chemotherapy (CT), but after initial responses, often relapses. Leukemia relapse is sustained by a subset of leukemia cells that are chemoresistant, and re-initiate the disease. Although cell-intrinsic factors are well-recognized players in chemoresistance, there is growing evidence that signals within the bone-marrow (BM) microenvironment imprint a “chemoresistant phenotype” to

otherwise chemosensitive cells. The CD44 adhesion molecule plays a crucial role in leukemic-cell homing to the BM. Alternative splicing of native transcripts generates different isoform variants, among which CD44v6. CD44v6 acts as a cytokine co-receptor, increasing the sensitivity to survival factors produced by the BM stroma, such as VEGF and scatter factor, the ligand for c-Met. Accordingly, CD44v6 over expression is associated with poor prognosis. We recently confirmed CD44v6 over expression in a relevant fraction of AML cases (11/26, 42%) with preference for M4-5 FAB subtypes, and interestingly in the majority of malignant plasmacells from multiple myeloma (MM) cases (15/17, 88%), another disease characterized by BM tropism. Primary AML cells cultured with BM-derived mesenchymal stromal cells (MSC) became relatively resistant to daunorubicin and ara-C. Acquired chemoresistance was cell-cell contact independent, suggesting the involvement of soluble factors released by MSC. SDF-1a was ruled out by blocking CXCR4 with plerixafor. Interestingly, while upon MSC co-culture the average levels of CD44 on AML cells decreased, we found that CD44v6 was selectively up-regulated. Accordingly, acquired chemoresistance could be reverted by blocking experiments with a mAb. Remarkably, the role of CD44v6 was confirmed in MM cells exposed to bortezomib. For *in vivo* proof-of-concept of acquired chemoresistance in NSG mice, we subsequently generated different AML and MM stable knock-downs specific for CD44v6 by LV-assisted short-hairpin (sh)RNA interference and surprisingly observed a virtually complete reduction of tumorigenic potential (P<0,005), rendering impracticable further experiments with CT. Altogether these results indicate that CD44v6 has a crucial and non-redundant role in those aspects of AML and MM cell biology that are microenvironment-dependent, including acquired chemoresistance and tumor initiation in xenograft models. Combining CD44v6 targeting with CT is therefore a promising approach for AML and MM eradication.

**P461**

**Prevention of xenogeneic graft-versus-host disease strengthens the engraftment of human acute myeloid leukaemia in NOD/SCID/IL2rgamma-/- mice**

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Xenotransplantation has become an indispensable tool in the exploration of stem cells and led to the discovery of cancer stem cells. Characterisation of CSC might provide insights in the pathophysiology of the disease, and also represents the basis to develop new therapeutic strategies. NOD/SCID/IL2rgamma-/- mice (NSG) provide a permissive environment for xenotransplantation and have been used to investigate leukemic stem cells of human Acute Myeloid Leukemia (AML).
Mononucleated cells (MNC) of 19 patients with AML were isolated from bone marrow, peripheral blood and leukapheresis products, and transplanted into unconditioned or sublethally irradiated NSG mice. Engraftment of human cells was subsequently monitored in the peripheral blood and finally bone marrow and spleen of the recipient mice were analyzed for the presence of human cells.
The majority of NSG showed a rapid expansion of human leukocytes in the peripheral blood, associated with deterioration of health. Sublethal irradiation enhanced the medical condition. The use of defrosted MNC mitigated the course of the disease, and the primary site of donor graft retrieval had no substantial influence on the engraftment. Engrafted human leukocytes cells were mainly polyclonal T cells. Furthermore, we observed the engraftment of human B-cells and cells of the myeloid lineage. Molecular analysis revealed that only cells of the myeloid lineage were derived from mutant primary leukemic cells. By transplanting MNC depleted of T- and B-cells, we could prevent an expansion of human T-cells. In those recipients, the engrafted leucocytes were constituted mainly of cells of the myeloid lineage, and only a minor population of B-cells was detected, strengthening the idea that expanding T cells are unrelated to the initial disease. This finding is supported by the observation that even transplantation of MNC of healthy donors in unconditioned NSG mice solely led to an engraftment of T-lymphocytes.
Transplantation of non-separated MNC of AML patients into NSG mice leads to the expansion of disease-unrelated T cells followed by a, most likely, T-cell mediated death of the mice. Therefore this transplantation procedure fails to establish an AML-like disease in the recipient mice, and is therefore not useful for the study of human AML in mice. We show here that T-lymphocyte expansion is circumvented by depletion of T cells in the donor material, which makes an engraftment of AML in recipient mice feasible.

## Infectious Complications

**P462**

**Incidence, risk and economic burden of viral reactivation after paediatric haematopoietic stem cell transplant**

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Objective and Methods: Reactivation of Epstein-Barr virus (EBV), Cytomegalovirus (CMV) and Adenovirus (ADV) is a major cause of morbidity after allogeneic haematopoietic stem cell transplant (HCT). From July 2005 to Dec 2010, 291 children (haematological=121, immunodeficiencies=154, metabolic=16) underwent HCT with prospective, twice weekly, surveillance for viral reactivation till CD4 recovery of 0.3 x $10^9$/l, and reconstitution of cellular immunity was tracked over a 12 month period. We have identified key risk factors for viral reactivation and provide a cost estimate in terms of the financial burden placed on the health care provider.
Results: Viral reactivation was frequent following HCT (CMV 17%, ADV 15% and EBV 11%) with the median time to onset of ADV viraemia 15 days, CMV 20 days and EBV 74 days after HCT. Children with reactivation had significantly slower kinetics of CD4 T-cell reconstitution compared to children without viraemia ($p<0.05$) with median CD4 lymphocyte counts under 150 x $10^6$/l in children developing CMV and ADV reactivations within 2 months, or EBV within 6 months of HCT. Use of serotherapy was a significant risk factor for all 3 viruses (Figure 1). In addition, CMV reactivation was significantly associated with donor/recipient seropositivity and use of mobilised peripheral blood stem cell grafts. ADV and EBV reactivations were significantly associated with HLA-mismatched grafts, acute graft versus host disease (GVHD) and extended use (>4 weeks) of steroid therapy. EBV was also linked to use of positive donor/recipient serology and reduced intensity condition regimens. Overall 9 (15%) of 62 deaths were directly attributed to one or more of these viruses. Children with reactivation remained in hospital for a significantly longer period (127 days *vs* 87 days; $p<0.01$) and most had additional post-HCT comorbidities, most notably GVHD. Extended hospital stay in children with GVHD ≥ grade II could be directly attributed to viral reactivation whereas for those with GVHD ≥ grade III the additional stay was multifactorial. We estimate the cost of antiviral therapy and additional in-patient stay at our centre attributable to viral reactivation is around 25,000 euros per patient.
Conclusions: Prospective surveillance of viral reactivation has allowed identification of high risk allogeneic transplant procedures in children, and for the first time we have been able to estimate the financial cost of viral reactivation to the health care provider.

[P462]

Fig 1 Cumulative total of CMV, ADV and EBV reactivations requiring antiviral treatment

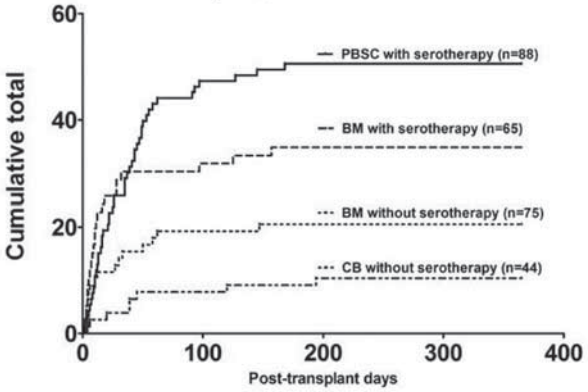


Fig 1 shows total number of CMV, ADV and EBV viral reactivations over a period of one year following transplantation with different stem cell sources with and without serotherapy

**P463**

**Tetanus, diphtheria and hepatitis B vaccination after paediatric cord blood transplantation**

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Background: The last updated unified international post-transplant vaccination guidelines recommends immunization of all patients at fixed times post hematopoietic stem cell transplantation (HSCT), independent of their stem cell source. It is not clear, however, whether this strategy will protect patients after cord blood transplantation (CBT) as they have a delayed immune reconstitution.
We aimed to assess the protection against diphtheria, tetanus and hepatitis B of pediatric transplant patients post CBT, at CHU Ste-Justine, Montréal.
Patients and Methods: The pediatric recipients of CBT at our institution, were tested for the presence of antibodies against 3 vaccine antigens: diphtheria, tetanus and hepatitis B. The vaccination schedule is composed of 3 doses of tetanus and diphtheria vaccine at 12, 15 and 24 months post-CBT, and 3 doses of hepatitis B vaccine at 12, 15 and 18 months post-CBT, according to the international guidelines.
Antibody-levels before vaccination (at 12 months following CBT) and after 2 and 3 doses of vaccination (at 18, 24 and 36 months post-CBT) were measured by ELISA assays.
Results: Twenty-eight CBT recipients were tested (20 males). The median age at CBT was 6.9 years (range 0.5 – 17 years). All the patients were vaccinated according to the immunization schedule excluding 2 patients who were not vaccinated before respectively 23 and 61 months post-CBT due to severe immune-suppression.
After 3 doses of vaccines, all patients had seroprotective antibody concentrations against tetanus and diphtheria. After the first 2 doses of vaccine, 60% of the patients had achieved protective antibody levels to tetanus and diphtheria.
For hepatitis B vaccination, 86% and 90% of patients achieved protective antibody-levels after respectively 2 and 3 doses of vaccination (samples obtained at 18 and 24 months post-CBT). However, at 36 months post-CBT, only 77% of them still had protective antibody concentrations against hepatitis B.
Conclusion: Despite the delayed immune restitution described in CBT recipients, vaccination against diphtheria, tetanus and hepatitis B results in seroprotective antibody concentrations. However, for hepatitis B, a loss of antibody response has been observed and suggests a long-term follow-up.

**P464**

**Diagnosis of invasive aspergillosis and mucormycosis guided by chest ct scan and PCR assays from bronchoalveolar lavage fluid in allogenic stem cell recipients**

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Patients face an increased risk of invasive mold infection, especially of the lungs during allogeneic stem cell transplantation (SCT). Low-dose CT-scan is a sensitive tool to detect lung lesions, and bronchoscopy with bronchoalveolar lavage (BAL) is a standard procedure to identify pathogenic microorganisms.
From April 2008 to August 2011 pulmonary infiltrates were detected by CT-scan in 64 adult patients (11,6% of all SCT patients) during allogeneic SCT. The BAL was examined by culture for pathogenic bacteria and fungi. Additionally, DNA was extracted and used in two semi-nested PCR assays targeting the mitochondrial DNA of Aspergillus spp. and the 18S ribosomal DNA of Mucorales, respectively. PCR products were characterised by sequencing.
In 28 (44%) out of the 64 patients fungal DNA was detected by the PCR assays in the first BAL. Sequencing revealed Aspergillus fumigatus specific DNA in 22 (78%) and A. flavus in one patient. Mucorales specific DNA was detected in 9 patients (32%), and identified as Rhizomucor spp in 6 and Rhizopus spp in 3 patients. A mixed infection according to PCR caused by A. fumigatus and Rhizomucor spp was found in 4 patients (14% of all fungal PCR-positives).
A follow-up bronchoscopy was performed in 22 of 64 (34%) patients. In the BAL of three previously PCR-negative patients A. fumigatus DNA was detected, and in one Rhizomucor spp

DNA was amplified in addition. In another patient A. fumigatus had been obtained in the first BAL but DNA of Rhizomucor spp was detected in the follow-up bal fluid.
Overall the PCR assays were positive in 31 (48 %) cases. Aspergillus spp. was detected in 20 (65%) patients, DNA of Mucorales spp in 5 (16%) and both A. fumigatus and Rhizomucor spp in 6 (19%) cases.
Out of 20 patients with detectable Aspergillus DNA 16 improved under antifungal treatment whereas 4 died. Out of 5 patients with Mucorales spp DNA only two survived whereas 3 out of 6 patients with a mixed infection survived, i.e. 21 patients (68%) with invasive fungal disease survived. In the fungal PCR-negative group consisting of 33 patients 23 (70%) survived.
In our hands the two PCR assays from BAL are a useful addition of the microbiological diagnostic armamentarium. It offers the opportunity to detect DNA from agents of mucormycosis. Pulmonary fungal infections are detected at an early stage in order to increase the chance of a successful antifungal therapy. Infiltrates during SCT are associated with a high case fatality rate and in many cases the etiology remains to be determined.

**P465**

**A comprehensive diagnostic approach improves the diagnostic accuracy of invasive fungal disease in adult haemato-oncology patients undergoing HSCT or high-dose chemotherapy- results of the King's Prospective Aspergillosis Study (NCT00816088)**

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Invasive fungal disease (IFD) is a difficult diagnosis. For clinical trials the revised EORTC/MSG criteria is useful but there is little data on its usefulness in clinical practice. The aim of this study was to evaluate the 'real world' incidence of IFD in patients undergoing HSCT or high dose chemotherapy using EORTC/MSG diagnostic tools. Patients were recruited prospectively between December 2008 and May 2010 and followed for at least 4 months after chemotherapy/HSCT. During admission twice weekly galactomannan (GM) and beta-D-glucan (BDG) was performed and neutropenic sepsis unresponsive to antimicrobials triggered diagnostic work-up with computed tomography (CT). The CT scans were reviewed independently by two chest radiologists and all cases were independently verified before assigning EORTC status. All patients had antifungal prophylaxis during the period of neutropenia or continuing immunosuppression. Two hundred and three patients were recruited [123 male, 80 female; median age 54y (range 19-73); median follow-up 194 days (range 12-647)]. The underlying diagnoses were: acute leukaemia 62 (myeloid 55, lymphoid 7), chronic leukaemia 4 (myeloid 3, lymphoid 1), MDS/MPD 33, aplastic anaemia 19, lymphoma 37 (non-Hodgkin's 29, Hodgkin's 8), multiple myeloma 45, and others 3. Main treatments received were: HSCT 165 (81%) [allogeneic 94, cord 5, autologous 66], chemotherapy 28 (14%), and immunosuppressive therapy (IST) 8 (4%). The total number of treatments received during study period was 263 (allografts 106, chemotherapy 77, autografts 67, IST 13). The patients were heavily pre-treated; 85% had at least one prior therapy with a median of 5 cycles of therapy/patient (range 1-17). There were 44 (21%) cases of IFD in 40 patients: proven 14 (Aspergillus fumigatus 2, Fusarium spp 1, mould IFD 5, non-candida albican spp 5, Pneumocystis jirovecii 1), probable 30. Invasive pulmonary aspergillosis was the commonest presentation (82%). The treatment-specific incidences were 17% (18/104), 12% (21/177), 7% (2/29), and 4% (3/72) for allogeneic HSCT, chemotherapy, immunosuppressive therapy, and autologous HSCT respectively. Median time to diagnosis of IFD was 42 days (range 3 to 524) from index chemotherapy or HSCT. Using galactomannan (GM) or beta-D-glucan (BDG) alone (plus host and CT scan evidence) the apparent incidence of IFD would be 13% and 17% respectively. Our findings demonstrate that a multi-diagnostic approach is necessary in order to improve diagnostic accuracy of IFD.

**P466**

**Mesenchymal stem cells suppress virus-specific T-cell responses *in vitro***

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Objectives: Viral infections, i.e. CMV and adenovirus (HAdV) and acute Graft-versus-Host disease (aGvHD) cause high morbidity and mortality in patients after hematopoietic stem cell transplantation (HSCT). Both complications can occur simultaneously and the risk of viral infections/reactivations is increased during steroid treatment.
Mesenchymal stem cells (MSC) are used for the treatment of steroid refractory aGvHD. On the other hand, adoptive T-cell therapy has been developed to target viral infections. We have investigated the interaction between MSC and virus-specific T-cells, because the effect of MSC on HAdV-specific responses is unknown and conflicting results have been published on the effect of MSC on CMV-specific responses.
Methods: PBMC were obtained from healthy volunteers and stimulated with HAdV or CMV peptivator (Miltenyi Biotec). PBMC were also stimulated with peptivator loaded autologous mature dendritic cells (mDC). To obtain virus-specific T-cell lines (TCL), cells were restimulated with autologous peptivator loaded PBMC on day 12 and 28. MSC were titrated in on day 0 (PBMC) and day 28 (TCL). Moreover, the effect of MSC on restimulated virus-specific T-cell clones was studied. Autologous EBV-B lymphoblastic cell lines were transduced with viral peptides for restimulation. As read-out, tritium-thymidine incorporation, IFN-gamma production, and flowcytometry (HLA-DR and CFSE) were applied.
Results: MSC suppress CMV peptivator stimulated, but not HAdV peptivator stimulated, PBMC. However, after stimulation with loaded mDC, the activation and proliferation of CMV and HAdV stimulated PBMC are suppressed. Responses of HAdV and CMV TCL restimulated on day 28 in the presence of MSC are suppressed. CFSE analysis generally shows that proliferation of CD8+ T-cells is more sensitive to suppression by MSC than the response of CD4+ T-cells (Figure 1). HLA-DR expression is also more suppressed on CD8+ T-cells. To further analyse possible differences in the effect of MSC on CD4 and CD8 T-cells, CD4+ HAdV and CD8+ CMV specific T-cell clones were studied. Both are not suppressed by MSC after restimulation. In control experiments, MSC also do not affect CD4+ or CD8+ minor HY specific T-cell clones.
Conclusion: MSC suppress HAdV and CMV peptivator induced T-cell responses *in vitro*. The clinical impact of this observation, i.e. during MSC treatment of aGvHD, is currently being investigated.

[P466]

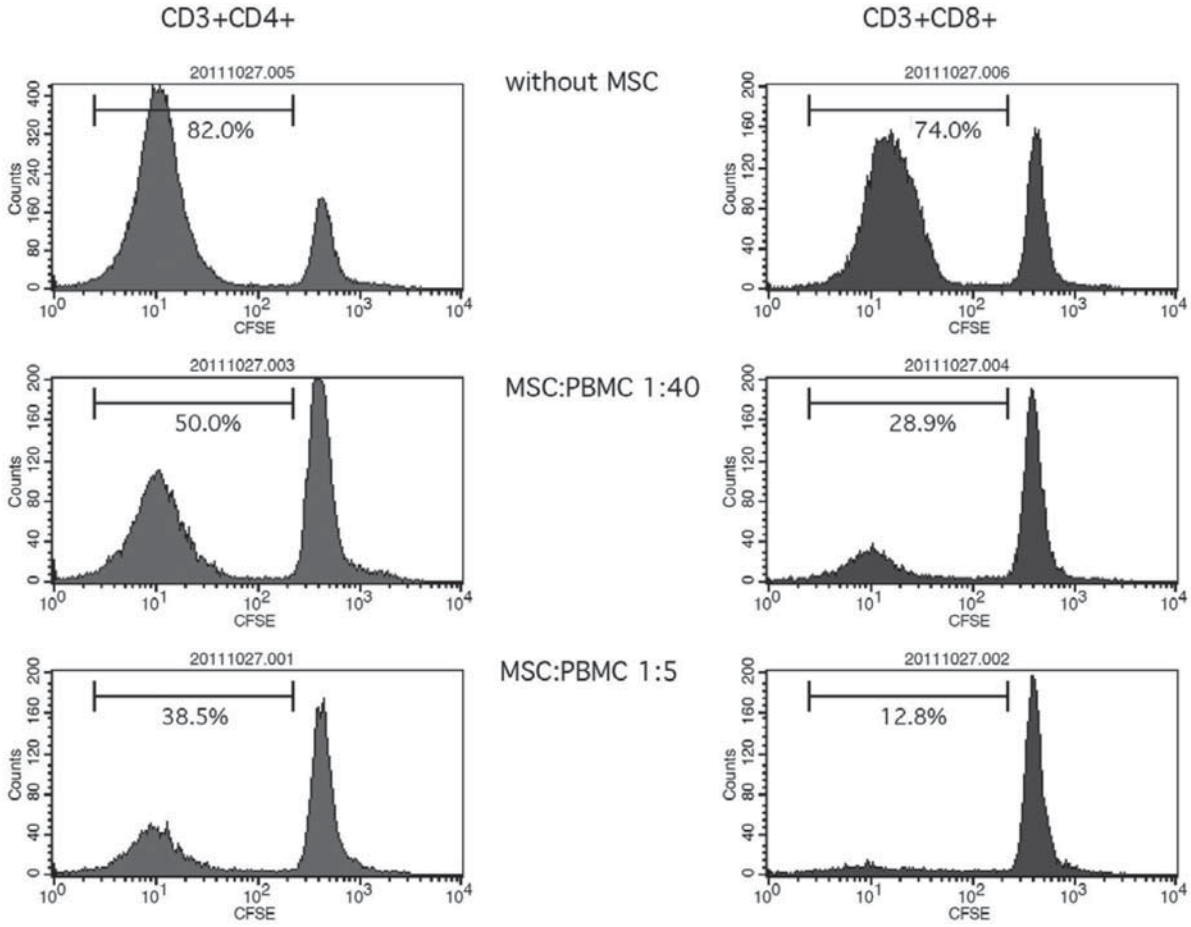


Figure 1. CFSE labelled PBMC were stimulated with CMV-peptivator for 7 days with or without MSC and analysed using flowcytometry. The percentage proliferating cells is depicted.

**P467**

**Multimer monitoring and ELISPOT analysis of antiviral T-cells in stem cell grafts. Effects of G-CSF mobilisation and the apheresis procedure on CTL frequency and functional activity**

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Cytomegalovirus-specific cytotoxic T cells (CMV-CTL) are routinely quantified in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) in our department to identify patients at high risk for CMV reactivation (CMV-R). Information on the number of CTL transfused with the graft and their fate is currently scarce.

Here, we analysed the number of CMV-, EBV- and ADV-CTL within the stem cell graft using multimer staining covering 6 common HLA class I molecules (A*01:01, A*02:01, A*24:02, B*07:02, B*08:01, B*35:01) to classify the donor's antiviral capacity and their potency to protect patients against CMV-R. Functional activity was tested by ELISPOT analysis after restimulation with overlapping peptide pools (CMV: pp65, IE-1; EBV: EBNA, LMP2A, BZLF; ADV: hexon). Antiviral-CTLs were analyzed in whole blood (WB) before and during G-CSF mobilisation (WBM), blood from the apheresis tubing filter (R) and from the graft (G). We aimed to detect a possible impact of G-CSF mobilisation as well as the influence of apheresis process on CTL frequencies and function. Up to date 42 donors were analyzed with multimers specific for each virus (7 CMV, 4 EBV and 3 ADV). In preliminary analysis the median levels of CMV-, ADV- and EBV-CTL varied depending on the multimer used for detection, but frequencies in an individual donor correlated well in WB, WBM, R and G samples. For example, median frequency of CMV-CTL detected with HLA-A*02:01-NLVP tetramer was 1.6% of CD3+CD8+ T-cells resulting in median numbers of 6/µl (n=28).

Interestingly, the number of CMV-CTL detected after G-CSF stimulation was not influenced by G-CSF, but function was impaired. Frequency of reactive T-cells detected by ELISPOT in samples collected after G-CSF stimulation (R, G) in an individual donor varied when compared to unmanipulated cells (WB). Furthermore, ELISPOT results did not correlate with the frequencies detected by multimer staining. This is currently under further investigation. Correlation of frequency and function of antiviral-CTL in the graft and reconstitution in the patient after HSCT will be analysed in a larger cohort.

Taken together, we suggest that accurate information on the numbers of transferred virus-specific CTLs and their functional activity should be determined to better understand the kinetics of antiviral reconstitution in patients after HSCT, to better identify patients in need of adoptive transfer and to select an optimal donor.

**P468**

**Candidate Immune markers for withdrawal of immunoglobulin replacement in allogeneic haematopoetic stem cell transplantation for malignancy**

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Background: Infection is a major cause of morbidity and mortality post allogeneic haematopoetic stem cell transplantation, allo HSCT. Immunoglobulin replacement, IVIG, is an effective strategy to enhance immune reconstitution and reduce infection risk but criteria for withdrawal of IVIG are not well established. In a retrospective study of a consecutive cohort we compared IVIG (12) versus no IVIG (19) cases using a standardised immune monitoring protocol.

Patients and Methods: 31 consecutive cases including 21 males, 10 females; age 43.2 + 13.4 years with recurrent infection and/or low serum immunoglobulins, Igs, (IgG <5 g/L n=11) were investigated. Underlying disease was ALL 8, AML 8, CLL 4, CML 3, NHL 4, HD 2, MDS 2. Donors were HLA-identical siblings in 17, and unrelated in 14. Donors and recipients were CMV negative in 13. Cases were referred within 1 year of HSCT (8), 1 - 2 years (8), 2 - 4 years (7), 4+ years (8), and compared with 27 age matched controls, 40.4 + 8.5 years. Immune monitoring included serum Igs; pneumococcal, tetanus, Haemophilus influenza b antibodies; major lymphocyte subsets; naïve, differentiated B and T cells; and T cell receptor repertoires, TCR.

Results: Cross-sectional study at first assessment revealed early recovery of B and CD8+ T cells but chronic deficits of NK, memory B and CD4+ T cells. Thymic naïve CD4+ T cells predict naïve B, memory B, and naïve CD8+ T cell counts, $r^2 = 0.481$, 0.367, 0.940, all $p<0.001$, reflecting combined immunodeficiency or immune reconstitution. Class switched memory B cells predict serum IgG, and IgA levels. Discriminating parameters were: serum IgG <5 g/L (not IgA, IgM, IgE, nor antibody levels) in 8/12 IVIG versus 3/19 no IVIG, $\chi^2$ 8.3, $p = 0.004$; memory B cells <15 x$10^6$/L in 9/12 IVIG versus 6/19 no IVIG, $\chi^2$ 5.55 p=0.018; and thymic naïve CD4+ T cells <10 x$10^6$/L in 9/11 IVIG versus 2/18 no IVIG, $\chi^2$ 14.50 p<0.001. CMV reactivation predicted CD8 counts; CD4, CD8 T cell phenotypes with loss of CD25, CD127, CD28 expression and TCR diversity, but not IVIG use. Serial study showed prolonged deficits of naïve T and memory B cells lasting ≥8 years post HSCT.

Discussion: IVIG is prescribed for symptomatic combined immunodeficiency of variable duration unpredictable by clinical criteria. Thymic naïve CD4+ T cells are discriminating indices strongly predictive of immunity, and with memory B cells are candidate immune markers for withdrawal of IVIG in allo HSCT for malignancy.

**P469**

**Extracorporeal photopheresis in patients with extensive chronic graft-versus-host disease does not lower risk of infection in the early post ECP period in spite of its steroid sparing effect**

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Immuno-suppression by glucocorticoids is the mainstay of treatment for extensive cGVHD. Infection due to Immunosuppression is the commonest cause of mortality in patients with cGVHD. In patients with cGVHD, ECP may down regulate allo-immunity and improve signs and symptoms of cGVHD, but the role of ECP in immune re-constitution and infection rate is not well studied.

We hypothesized that ECP in patients with cGVHD may allow tapering of corticosteroid dose which in turn may alter the risk of infections in the post ECP period.

In an attempt to test this hypothesis, we retrospectively reviewed the charts of 18 consecutive patients (median age 51 years; range 24-69; M: F 10:8) with extensive cGVHD starting ECP at our institution and prospectively followed for three years (2007-2010). Leukemia/MDS (n=16) was the most common indication for transplantation. Thirteen patients (72%) received matched related donor and 5 patients (28%) received matched unrelated donor transplantation. Median time of onset of cGVHD after transplantation was 24 months (range 5-60 months). Median time to start ECP after diagnosis of chronic GVHD was 10 months (range 0-60 months). All patients (100%) were on prednisone when ECP was started. Prednisone dose could be decreased in 15 patients (83%) after initiation of ECP. ECP and steroids could be stopped altogether in 3 patients (17%) after a median of 18 months. 7 of the 13 (53%) patients suffered from severe infections necessitating hospitalization prior to initiation of ECP. Eleven of the 18 patients (61%) needed hospitalization secondary to infection during or after completion of ECP. Majority of the infections necessitating hospital admission prior to initiation of ECP were Pneumonia (n=6), sepsis (n= 3), and port infection (n=1). Infections necessitating admission in patients during and after completion of ECP were Pneumonia (n=10), port infection (n=3), and wound infection (n=3). Some patients had multiple infections.

In this small cohort we observed, that majority of patients during ECP could lower the dose of prednisone and 3 patients could altogether stop both ECP and steroids. Though ECP led to decreased steroid use, it did not change admission rates due to severe infection indicating ongoing immune deficiency. Prospective trial in a larger cohort and detailed monitoring of immune re-constitution assays will be necessary to show if ECP truly affects spectrum and incidence of infection in patients with extensive cGVHD.

**P470**

**Prognostic factors and survival in patients receiving haematopoietic stem cell transplation admitted to an intensive care unit**

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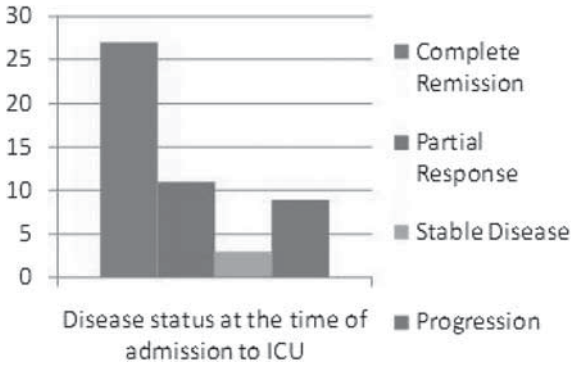
Introduction: Intensive Care Unit (ICU) admission following haematopoietic stem cell transplantation (HSCT) is controversial due to the limited prognosis of these patients in case of secondary critical illness.

Aim: To evaluate prognostic factors and outcomes of the highest risk subgroup HSCT recipients requiring admission to ICU.

Patients and Methods: Retrospective study of patients admitted to the ICU after HSCT between January 2000 and April 2011 at anytime after allogeneic HSCT and until day +100 for autologous HSCT. We collected demographic data, comorbidities, disease characteristics, reason for ICU admission and survival (hospitalary and after one year). SOFA and SAPSII scales were calculated and used as prognostic scores.

Results: 50 patients undergoing HSCT were admitted to the ICU, 80% (n=40) received allogeneic HSCT. The median age was 40 years (range 11-69). 56% were men. The underlying disease was: 51% acute leukemia, 23% lymphoproliferative syndrome and 26% myelodysplastic or myeloproliferative syndromes. The status of hematological disease at the time of admission is detailed on Graphic 1. Among allogeneic HSCT patients, 37.5% presented acute graft versus host disease (GVHD), and 30% chronic GVHD. The most common reasons for admission were sepsis, respiratory failure and cardiac failure in both allogeneic (35, 40 and 17% respectively) and autologous (60, 30

[P470] Graphic 1. Disease status at the time of admission to ICU




and 10%) HSCT. 62% required non invasive mechanical ventilation (NIMV), 50% invasive MV (IMV) and 72% vasoactive drugs. The hospital survival was 40% and 29% after one year. The presence of acute GVHD (p=0.041), the reason for admission (p=0.041), the requirement for NIMV or IMV (p<0.001), the need of vasoactive drugs (p=0.002) and higher scores on scales SAPSII and SOFA (p<0.001) at the time of admission were associated with higher mortality rate in the univariate analysis. The only predictor of mortality in the multivariate analysis was SOFA score (p=0.023).
Conclusions: The most frequent reasons for ICU admission were sepsis and respiratory and cardiac failure. The presence of acute GVHD in the allogeneic patients and the requirement of respiratory support, vasoactive drugs and higher SAPSII and SOFA scores were significantly associated with lower hospital survival. The main prognostic was the SOFA scale score at time of admission, a marker of multiple organ failure. The ICU admission had presented a contribution to the survival of 40%.

**P471**
**Characteristics and outcomes of cytomegalovirus infection in 115 patients undergoing allogeneic haematopoietic stem cell transplation in a single centre**
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Cytomegalovirus (CMV) infection and graft versus host disease (GVHD) are the most important determinants of morbidity and mortality after allogeneic hematopoietic stem cell transplant (HSCT). Different factors are associated with the development of CMV infection in these patients.
Aim: To analyze the incidence of CMV infection in patients undergoing allogeneic HSCT in our center and its impact on GVHD, relapse of the underlying disease and survival.
Patients and Methods: Retrospective study of 115 consecutive patients undergoing allogeneic HSCT between January 2005 and January 2011. We collected demographic data, donor (D) and receptor (R) CMV serostatus, conditioning regimens, GVHD prophylaxis and clinical outcomes from patient's charts. Information of weekly test for reactivation of CMV by antigenemia or quantitative PCR was also collected.
Results: 53% received reduced intensity conditioning. 68% received a graft from a related donor (RD). GVHD prophylaxis was cylosporin + methotrexate in 82% of RD cases. Patients receiving HSCT from unrelated donor (URD) received different GVHD prophylaxis regimens, including pretransplant rabbit thymoglobulin in 49% of cases. The stratification of risk for reactivation of CMV according to D/R serostatus was: low (D-/ R-) in 6% of cases, intermediate (D+/R-) in 49%, and high risk (D±/R+) in 45%. We observed 40 CMV reactivations, with a median onset of day +52 post-HSCT (range 20-205). 3 patients (2.6%) developed confirmed CMV disease. The cumulative incidence of CMV reactivation, GVHD and relapse at day +100 were 27%, 44% and 5% respectively. In the multivariate analysis, donor type (URD *vs* RD) and the development of acute GVHD remained as independent factors for reactivation of CMV (p <0.001 and p=0.002 respectively). There was no significant association between CMV reactivation incidence and CMV D/R serostatus, probably related to the small size of our series. All patients received preemptive antiviral therapy. With a median follow up of 23 months (0-73), 50% of patients who presented a CMV reactivation died (68% of them due to non CMV infection causes, 32% of non-infection causes) compared to 36% mortality rate among patients who did not present CMV reactivation (p 0.04). Overall survival of the series was 60%.
Conclusions: This study confirms the higher risk of CMV infection among patients receiving transplant from URD and among those who develop GVHD and the impact of the CMV reactivation on mortality rate.

**P472**
**Infection-related deaths in the haematopoietic stem cell transplant setting**
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Background: For decades infections have been among the major causes of death in patients undergoing hematopoietic stem cell transplantation (HSCT). In the era of novel diagnostic and therapeutic strategies, outcome variables should carefully be monitored and re-evaluated.
Methods: In a single-center, retrospective survey data from a total of 607 adult and pediatric patients (242 allogeneic and 365 autologous graft recipients) undergoing HSCT between 2008 and 2010 were analyzed. An autopsy was performed in all fatal cases. Pathogens were identified on morphological basis and culture. No nucleic acid based tests were used on tissue specimens. A possible infectious cause of deaths was investigated based on post mortem and clinical findings. In cases of most prevalent infections the time to death post transplantation was also calculated. In mold infections results were compared to historical control data.
Results: During the median follow-up time of 113 (1-891) days 77 (16 autografted and 61 allografted) patients died. In the autologous transplant population only 3 patients died of infection

(bacterial: 1, mold infection: 2). Both of these mold infection cases were heavily pretreated multiple myeloma patients dying of an invasive aspergillosis. In the allogeneic HSCT setting 33 fatalities (55% of deaths) could be related to infection. Mold: 16 (25%), bacterial: 9 (15%), viral: 7 (11%), protozoon: 1 (2%) and mold+viral double infection: 1 (2%). Compared to historical control (same centre, between 2003-2006) a non-significant decrease in mold related deaths could be observed (7 *vs.* 8.2 % of all allogeneic HSCT cases). While the rate of invasive aspergillosis-related deaths decreased, a minor increase was seen in fatalities due to mucormycosis (4.5 *vs*. 6.1%, and 2.5 *vs.* 2% of all allogeneic HSCT cases, respectively). Time to death due to aspergillosis after HSCT was significantly shorter when compared to mucormycosis (mean 98.8 *vs*. 178.5 days, p=0.05).
Conclusions: 1. Infectious mortality remains low in autologous HSCT but infection is still a leading cause of death in the allogeneic HSCT setting. 2. Invasive mold infections can occur in pretreated and autografted multiple myeloma patients. 3. The incidence of fatal invasive aspergillosis slightly decreased in allogeneic HSCT but the rate of mucormycosis showed a tendency to increase. 4. Fatal aspergillosis occurred significantly earlier post transplantation than deaths due to mucormycosis.

**P473**
**Reduced-intensity conditioning reduces the risk of early death from pneumonia following haematopoietic stem cell transplantation**
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Reduced-intensity conditioning (RIC) has been shown to reduce transplant related mortality after HSCT compared to myeloablative conditioning (MAC). We performed a retrospective cohort study to find out whether the use of RIC might also contribute to reducing the risk of early death from pneumonia.
Pneumonia-associated deaths were evaluated in 691 HSCT patients. The majority had a hematological malignancy (n=504) and an HLA-matched donor (n=584). RIC was given to 336 patients and MAC to 355. Data concerning radiology, laboratory values, culture, and autopsy results were evaluated together with risk factors for death related to pneumonia within or after 100 days after HSCT (early and overall pneumonia) was evaluated.
In 68 patients pneumonia contributed to death (early n=22). The incidence of early pneumonia-related death was 4.2% and 2.1% in MAC and RIC patients, respectively. The overall incidence of pneumonia-related death was 10.5% in both groups.
In the multivariate analyses, a malignant disease, a previous HSCT, acute GVHD of grades II-IV, bacteremia and having received mesenchymal stromal cells (MSCs) were factors independently associated to overall pneumonia-related death. MAC-regimen, bacteremia and having received a previous HSCT were found to be associated with early pneumonia-related death.
Conclusions: RIC reduced the incidence of early death associated with pneumonia. Patients receiving MSC may have an increased risk for pneumonia-related death. Pneumonia is still an important contributor to overall death in HSCT patients.

**P474**
**Effect of smoking history on the risk of pneumonia during aplasia and engraftment following allogeneic stem cell transplantation**
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Pneumonia is a frequent complication of aplasia and engraftment following allogeneic stem cell transplantation (alloSCT). This retrospective analysis was aimed at understanding whether a positive smoking history may increase the risk of pneumonia during aplasia and engraftment.
We collected from clinical charts full data about pulmonary infections during aplasia and engraftment of all the patients consecutively allografted at National Tumor Institute of Milan, Italy, from 2004 to 2011. Smoking history data were collected from clinical charts or by telephone interview. Pneumonia was diagnosed by chest X-ray with associated signs of lung infection, respiratory symptoms and/or fever. High resolution CT scan of the lungs was confirmatory whenever radiographs were unsatisfactory for diagnosis.
Patients evaluable were 170. Their median age was 49 years (range, 15-66 years), and they received alloSCT for lymphoma (57%), myeloma (26%), or acute leukemia or myelodysplasia (17%) from HLA identical siblings (43%), matched unrelated (45%) or haploidentical donors (12%). Eighty patients (47%) had a positive smoking history, with a median consumption of 1.0 pack of cigarettes a day (range, 0.1-3.0 packs a day) for a median of 12.0 years (range, 1.0-40.0 years). Seventy-one patients had quit smoking a median of 5 years before alloSCT (range, 0.5-41.0 years), 9 patients were still smokers at transplant. Smoking habit was more frequent among males (p=0.005) and among patients living their 5th to 6th decade than younger ones (p<0.001). Smokers had more frequently than nonsmokers a significant reduction (>20%) of forced expiratory volume in 1 second (FEV1) and diffusion lung capacity for carbon monoxide (DLCO) corrected for hemoglobin (p<0.001 and p=0.004, respectively). During aplasia and engraftment, 24 patients (14%) were diagnosed with pneumonia by chest X-ray (22 positive exams) and/or lung CT (14 positive exams), with associated fever (22 patients) and/or respiratory symptoms (9 patients). Twelve of 80 smokers and 12 of 90 nonsmokers developed pneumonia (15% *vs*. 13%, p<0.001), with an absolute 2% incidence increase and a 13% estimated increase of risk of pneumonia solely attributable to smoking habit.
In conclusion, smoking history significantly increases the risk of pneumonia during aplasia and engraftment following alloSCT. Prospective studies are needed to understand whether respiratory physiotherapy or a tailored antibiotic prophylaxis may decrease this risk.

**P475**
**Comparison of the effects of +3 day post-transplant daily filgrastim doses versus a single pegfilgrastim dose in autologous SCT**
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Background: The use of high-dose chemotherapy followed by autologous stem cell transplantation is an important treatment option for selected patients with hematological malignancies. Hovewer, the high chemotherapy doses used for pretransplantation preparation exposes patients to the risk of neutropenic complivations, including bacterial and fungal infections,that in rare cases can be fatal. The post-transplant administration of filgrastim reduced the time to neutrophil recovery and has therefore become standard practice in many institutions. Alternatively, the long-acting filgrastim formulation, pegfilgrastim, can be administered as a single 6 mg dose and has a significantly increased half-life, partly due to a decreased renal clearance.
Patients and Methods: In this study, data of 72 consecutive adult patients who received an auto-SCT between January 2009 and May 2011 and filgrastim (40pts) or pegfilgrastim (40pts) after transplantation were retrospectively examined. Diagnoses were non Hodgkin lymphoma (26 pts), Hodgkin lymphoma (24 pts) and Multiple Myeloma (30pts). Standard conditioning regimens (HD-Melphaln or BEAM) were used. The mean CD34+ stem cell doses infused were 4.1 and $4.9 \times 10^6$/kg (p=ns) for the

pegfilgrastim and filgrastim group; the groups were matched for age, sex and underlying disease. Patients of the filgrastim group received daily subcutaneous injections of 5 mg/Kg/day starting at the day +3 after transplantation until ANC >1x10$^9$/L; patients of the pegfilgrastim group received a fixed dose of 6 mg subcutaneously on day +3 post-transplantation.
Results: The median time to an ANC of 0.5x10$^9$/L was 9 and 10 days (p=0.04), respectively, in the pegfilgrastim and filgrastim groups. There was no significant difference in the platelet engraftment between the pegfilgrastim and filgrastim groups (11 *vs* 12 days, respectively, p=ns). The median number of days with febrile neutropenia in the pegfilgrastim group was 2 (range 1-5), versus 3 (range 1-6) in the filgrastim group (p=0.09). There was no difference in the incidence of documented infections (22% in the pegfilgrastim *vs* 25% in filgrastim, p=0.8). Median hospital stay (from day 0) was 15 day for pegfilgrastim and 16 days for the filgrastim group (p=0.2); there was no significant differences in survival at day +100 or at 1 year.
Conclusions: We conclude that a single injection of pegfilgrastim administered on post-transplant day+3 shows comparable safety and efficacy profiles to +3 daily injections of filgrastim.

**P476**
**Early post-transplant infections in AML patients conditioned with Bu-Cy regimen: a 10 year single-centre experience**
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Introduction: Infections are the most important complications after stem cells transplantation. The aim of this study is to evaluate incidence, type and severity of infective complications during the early post-transplant period (from day 0 to day +30) in patients with acute myeloblastic leukemia (AML), conditioned with Bu-Cy chemotherapeutic regimen. We perform comparative study between autologous and allogeneic recipients.
Material and Methods: During a ten years period (2000-2010) we have treated 110 AML patients with stem cell transplantation. Male: 58 Female: 52 Median age: 34 years. Autologous:70 Allogeneic from HLA identical sibling: 40, peripherall stem cells: 90, bone marrow: 20. Myeloablative preparative regimen Busulfan/Cyclophosphamide was used in 90 patients (60 autologous; 30 allogeneic). Patients were treated in sterile room conditioned with HEPA filters, low bacterial diet and antiinfective prophylaxis regimen consisted Ciprofloxacin 1000 mg/day; Fluconazole 200 mg/day, or Itraconazole 200 mg/12 h; Acyclovir 250 mg/8 h). $\chi^2$ was used to compare frequency of infective complications in autologous and allogeneic transplantation.
Results: In the group of allogeneic recipients 20/30 patients (66,6%) developed fever, mucositis gr III/IV 6/30 (20%), pneumonia 5/30 (16,6%), acute viral B-hepatitis 2 patients (2,2%), thrombophlebitis 3/30 (10%), central venous catheter associated infection in 6/30 (20%), The most frequent isolated microorganisms were Gram-positive cocci (70%), Gram-negative bacilli (20%), Candida albicans (6%), Non-albicans Candida (4%). One patient die in septic shock caused by Pseudomonas aeruginosa (6,6%). In the group of autologous AML recipients febrile episode were noted in 30/60 patients (50%), mucositis gr.III/IV 6/60 (10%), pneumonia in 5/60 (8,3%). The most common isolated were Gram-positive cocci (65%), Gram-negative bacilli (25%), Non-Albicans Candida (5%), Candida albicans (5%). The infective complication was fatal in one patients due to septic shock caused by Streptomonas maltophilla.
Conclusions: In our center confirming other study data we detected no significant differences between AML patients treated with autologous or allogeneic stem cell transplantation (preparative regimen BuCy) with respect to infectious complications (p=0.118).

**P477**
**Incidence and nature of early infections in children following allogeneic haematopoietic stem cell transplantation from matched family donor, with impact on procedure-related mortality: a single-centre experience in a developing country**
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Objectives: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has become a definitive therapeutic modality in children. Numerous advances have taken place to make the procedure safer. However, infectious complications remain a major cause of transplant related morbidity and mortality. Early infections and infection-related mortality are defined as those occurring within 100 days after transplant. In developing countries, early infectious complications are expected to be higher because of the newly settled centers, performing fewer transplants per year, having fewer educated nurses and staff, insufficient isolation facilities and more numbers of constructions especially making the risk of invasive fungal infections (IFIs) higher. The aim of this study was to find out the incidence and nature of early infections and attributable mortality following allo-HSCT from matched family donor (MFD) for hematologic disorders performed in a single pediatric institution in a developing country.
Methods: Medical records of all consecutive myeloablative allo-HSCTs from MFD performed for malignant or non-malignant hematologic disorders from November 1997 through December 2010 were retrospectively reviewed to find out the frequency and etiology of early infections, and the incidence of early infection-related mortality.
Results: A total of 120 patients underwent 122 HSCTs. Underlying disease was malignant in 48 (39%) and non-malignant in 74 (61%). A total of 170 infection episodes developed within 100 days after transplants. Seven (5.7%) of all patients died. Infections (28.5%) were the second most cause of early mortality following veno-occlusive disorder (43%). Incidence of infection-related early mortality was 1.6% (2/122). Nine (7.3%) of all patients had IFI and one of them (11%) died.
Conclusions: In our center which is showing our country's characteristics, the incidence and nature of early bacterial and fungal infections were similar to developed countries' results. The incidence of infection-related early mortality and the role of infections in this mortality were lower when compared with developed countries' data performed on same time periods with similar transplant characteristics. Furthermore, the frequency

**[P477]** **Table 1. Underlying diseases in 122 HSCTs**

| Underlying disease | (Number, %) |
|---|---|
| TM (Class MI) | 21(17) |
| TM (Class III) | 33(27) |
| SCA | 1 (1) |
| AA | 6(5) |
| FA | 13(11) |
| AML | 25(21) |
| ALL | 5(4) |
| MDS | 8(6) |
| CML | 6(5) |
| HLH | 4(3) |

of IFIs and IFI-related mortality were not higher also. In conclusion, the incidence of early infectious mortality following allo-HSCT may not be different in developing countries by means of universal preventive strategies and attentive care, despite insufficient conditions by a dedicated team.

**P478**

**Is procalcitonin useful in the management of stem cell transplantation?**

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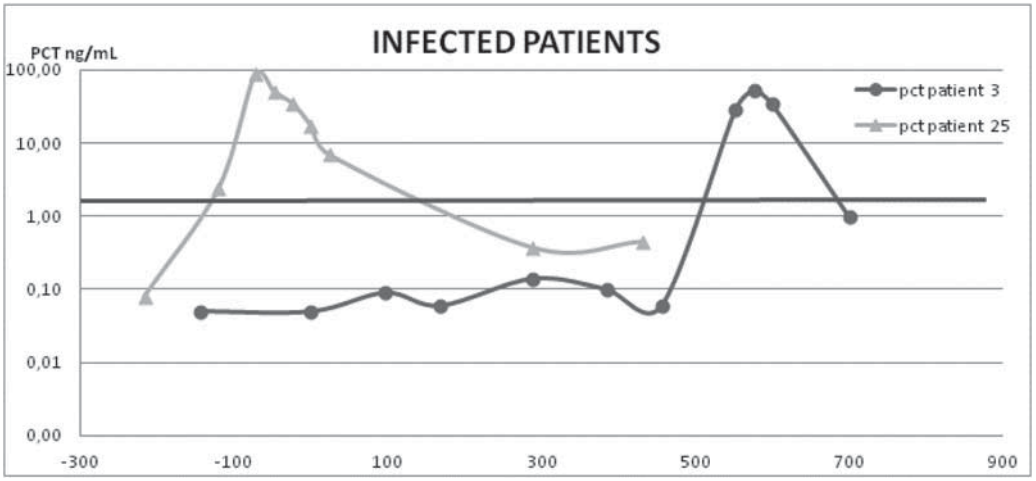
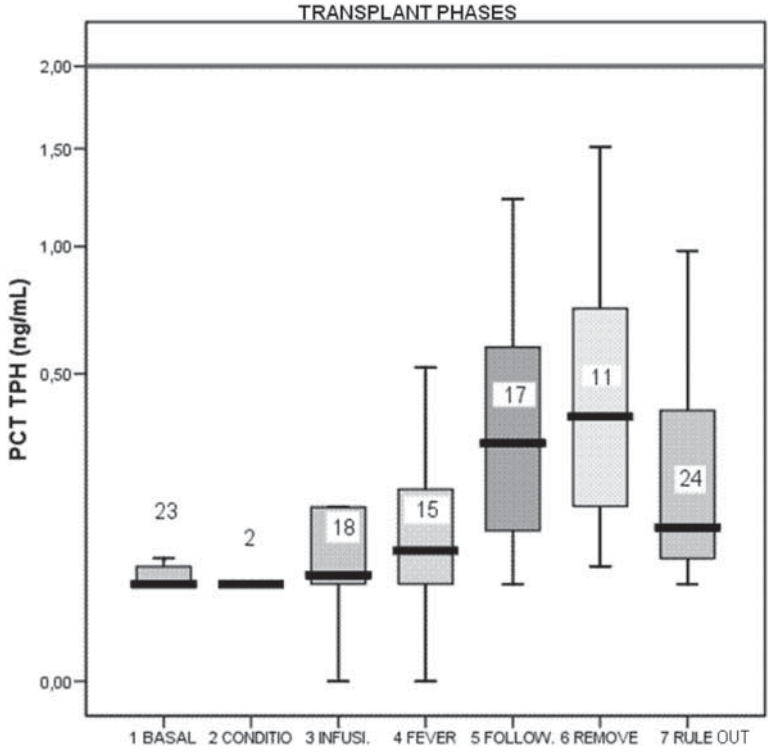
Introduction: Procalcitonin (PCT) is a prohormone that is produced in bacterial infection, like C reactive protein (CRP), although the latter has a low specificity. The clinical utility of PCT is not well known in severe immunosuppressant conditions, such as stem cell transplantation (SCT).
Objectives: Finding PCT concentrations and their predictive value in the diagnosis of severe bacterial infection in SCT compared to CRP levels.
Methods and Materials: Prospective study including 31 patients with SCT from March to October 2011. PCT and CRP concentrations were obtained the day of admission, during conditioning, the day of stem cell infusion, and in the main post-transplantation events (fever, antibiotic suspension and discharge). Follow-up was carried on until day +90 from SCT.
Results: A total of 136 samples from 25 patients were analyzed, with a mean value of 5 samples per patient. In the conditioning period 3 patients fulfilled sepsis clinical criteria, with PCT concentrations above the cut-off level (2ng/mL) with a median value of 11.08ng/mL. Excluding infected patients, during SCT, median PCT level was of 0.11 ng/mL (p90: 0.862) (cut-off value

**[P478]**



2 ng/mL) and median CRP level was of 29.4 mg/dL (p90:163.02) (cut-off value 5mg/dL). During the follow-up, two types of pattern were found: patients that reached values of 0.20 (n= 14), and other patients who scored up to 0.60 (n= 11). In all of them CRP levels were very high (median 35.80 mg/dL p90: 161.9). No significant differences were found in PCT concentrations based upon type of transplant, induction or haematological disease. Only 3 patients were analyzed during the post-transplantation period due to febrile episodes. Two of them did not exhibit PCT elevation (although CRP levels did rise) and were discharged in less than 6 hours. The third patient, who met clinical criteria for sepsis with Acinetobacter isolated in blood cultures, achieved PCT values of 28.8, 53.38 and 33.59 ng/mL and CRP levels of 30, 84.9 and 74.2 mg/dL.
Conclusions: In patients without infection, PCT levels remained always below pathological values. However, CRP remained elevated in many of them. We can conclude that in septic situations, in conditioning, during transplantation and after transplantation, PCT levels increased significantly and was useful both in the diagnosis and follow-up of sepsis in SCT.

**P479**

**At home treatment after high-dose chemotherapy and autologous stem cell transplantation is safe and feasible. Evaluation of 4 years of ambulatory care from a medical, nursing, patient and financial perspective**

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The necessity of keeping patients in hospital during neutropenia after high dose chemotherapy is under discussion. A prospective, nonrandomized clinical study was done to examine the safety of ambulatory care in patients undergoing consolidation chemotherapy for acute leukemia, or autologous stem cell transplantation for lymphoma or multiple myeloma. Patients fulfilling the eligibility criteria were discharged into ambulatory care the day after the last chemotherapy administration, or the day after reinfusion of the stem cells. Patients visited the ambulatory care unit 3 times a week for monitoring of signs, symptoms and laboratory results.
During the study period, 165 patients were admitted for 208 chemotherapy cycles. 76 patients in 89 cycles could not be included in the ambulatory care program, most frequently either because their medical situation did not allow for early discharge (58%), or because they had no care giver (14.6%), or had to travel a large distance to the hospital (14.6%).
The 89 patients in the ambulatory care group, who underwent 119 cycles of high dose chemotherapy, spent almost 70% of the neutropenic phase at home. 37 out of 89 patients (46 cycles) were never readmitted to the hospital. None of these patients had to be admitted to the intensive care, and there was no treatment related mortality In the hospital group, 2 patients died, one because of pneumonia and one because of invasive aspergillosis.
Patients and their caregivers felt safe and comfortable at home, and the vast majority preferred home care over in-hospital treatment.
This study demonstrates the safety and feasibility of managing carefully selected patients in an ambulatory care setting after high dose chemotherapy.

**P480**

**Invasive aspergillosis in adult haematopoietic stem cell transplant recipients**

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Background: Invasive aspergillosis (IA) is major cause of morbidity and mortality in hematopoietic stem cell transplant (HSCT) recipients. This study focuses on the risk factors, etiology and outcomes of IA.
Methods: 356 pts after allogenic (allo) (237) and autologous (auto) HSCT (119) were included in 2000-2010 yy. Baseline patient characteristics and abbreviations are outlined in Table 1. EORTC/MSG 2008 diagnostic criteria of IFD (proven and probable) were used.
Results: The incidence of IA in alloHSCT group was higher 19% (45/237) then in autoHSCT group 9,2% (11/119) ($p<0,001$). Etiologic agents of IA after alloHSCT were A. fumigatus 60%, A. niger 20%, A. flavus, A. ochracea, A. terreus – 9%, and unidentified Aspergillus spp. 11%. IA after autoHSCT were caused by A. fumigatus 54,5%, A. niger 18,2%, A. flavus 9,1%, unidentified Aspergillus spp. 18,2%. Median date of IA onset after alloHSCT was D+34 (3-610), after autoHSCT – D+11 (6-38) ($p<0,05$).
In alloHSCT group risk factors were: previous IFD, non-myeloablative (RIC) regimen, fludarabin and anti-thymocyte globulin (ATG) use in conditioning regimen, lymphopenia >3 weeks, grade 4 neutropenia >2 weeks, acute GVHD, severe bacterial infection, CMV infection, underlying AL, and PBSC as a source of HSC ($p<0,05$). In this group cyclosporine A use was associated with higher risk of IA than tacrolimus ($p<0,05$).
In autoHSCT group risk factors were: previous IFD, mucositis grade III-IV, lymphopenia >3 weeks, grade 4 neutropenia >2 weeks, severe bacterial infection ($p<0,05$). In this group G-CSF use was associated with increased risk of IA ($p<0,001$).
In HSCT recipients the 12-week OS rate after diagnosis of IA was 68,2%. In 2000-2005 yy. 12-week OS rate was 52,6%, in 2006-2010 yy. – 72,7% ($p<0,05$) due to routine diagnostic procedures (galactomannan (GM) test and CT scan) and new antifungal drugs use.
Conclusion: The incidence of IA in HSCT recipients was 16% (allo – 19%, auto – 9%). We reveal high rate of Aspergillus non-fumigatus as etiological agents. Usage of new antifungal drugs, routine GM test and CT scan improved 12-week OS rate.

**[P480]** **Table 1. Baseline patient characteristics for 356 hematopoietic stem cell transplant (HSCT) recipients.**

| Variable | Allogenic transplant (n = 237) | Autologous transplant (n = 119) |
|---|---|---|
| **Demographic characteristic** | | |
| Age, years | | |
| Median, range | 27 (18-66) | 33 (18-67) |
| Sex | | |
| Male/Female | 150/87 | 47/72 |
| **Underlying disease** | | |
| Diagnosis | (%) | (%) |
| Acute leukemia (AL) | 151 (63.7) | 9 (7.7) |
| Acute myeloid leukemia (AML) | 71 (47) | 6 (6.6) |
| Acute lymphoblastic leukemia (ALL) | 80 (53) | 3 (3.3) |
| Chronic leukemia | 29 (12.3) | - |
| Lymphoma | 25 (10.5) | 62 (52.1) |
| Multiple myeloma | - | 34 (28.6) |
| Myelodysplastic syndrome | 16 (6.8) | - |
| Aplastic anemia | 12 (5) | - |
| Other | 4 (1.7) | 14 (11.7) |
| Status at the moment of HSCT | | |
| Remission | 124 (52.3) | 87 (73.1) |
| Relapse | 113 (47.7) | 32 (26.9) |
| **Transplant characteristic** | | |
| Hematopoietic stem cell (HSC) source | | |
| Bone marrow (BM) | 115 (48.5) | 32 (26.9) |
| Peripheral blood (PBSC) | 115 (48.5) | 79 (66.4) |
| Combination of BM and PBSC | 7 (3) | 8 (6.7) |
| Receipt of conditioning | | |
| Myeloablative (MAC) | 124 (52.3) | 119 (100) |
| Non-myeloablative (RIC) | 113 (47.7) | - |
| **Transplantation complications** | | |
| Graft-versus-host disease (GVHD) | (%) | (%) |
| Acute | 20 (8.4) | - |
| Chronic | 35 (14.8) | - |
| Mucositis, grade 3-4 | 62 (26.2) | 21 (17.6) |
| Cytomegalovirus infection (CMV) | 98 (41.3) | - |
| Bacterial infection | 49 (20.7) | 11 (9.2) |
| Receipt of granulocyte colony-stimulating factor (G-CSF) | 64 (27) | 32 (26.9) |

**P481**

**Invasive aspergillosis following haploidentical haematopoietic stem cell transplantation. A single-centre experience**

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Invasive aspergillosis (IA) is a major cause of morbidity and mortality among patients undergoing HHSCT. The aim of our study is to identify factors related to the occurrence and the outcome of IA in this patient cohort. Between 2005 and 2010, 33 (M:F 22:11) patients received a HHSCT. Their median age was 52 years (range 17-69). All patients were heavily pre-treated (12 had previously undergone autologous transplantation) due to primary resistant haematological malignancies and/or early disease relapse. Four patients had a previous invasive fungal infection. Graft donors (M:F 27:7) had a median age of 41 years (range 21-67). High resolution HLA typing was available for 28 cases. Among them, in 12, the donor–recipient HLA combinations predicted NK cell alloreactivity against the HLA type of the patient. All conditioning regimens were Fludarabine based. All patients received peripheral blood stem cells. Eight grafts were obtained after selection of CD34+ cells, 24 after depletion of CD3+/CD19+ cells and 1 after both procedures. The median number of cells was: CD34+ 5.33E+06/Kg, CD3+ 3.8E+04/Kg, CD19+ 3E+04/Kg, NK 2.3E+08/Kg. Seventeen patients received prophylaxis against aGvHD. Six developed aGvHD grade II-III and 27 grade 0-I. The data of the HHSCT were compared to those from 39 patients who received graft from a matched sibling donor (control group).

The median survival in HHSCT recipients was 115 days (range 3-1198). Data for the occurrence of IA were available for 21 out of 33 patients. Based on the EORTC criteria, 15 cases were characterized as negative or possible and 6 as probable or proven (28%). Between these 2 groups there was no significant difference on survival, on acute GvHD development and on the presence of KIR alloreactivity. The numbers of NK and T cells in the graft were not significantly different. However, it was observed a trend of a higher number of CD34+ cells correlated with a lower incidence of IA ($p < 0.1$). A lower incidence of IA was observed in the control group (5 probable cases, 12%). Interestingly, the occurrence of IA in the later was correlated with a reduction of survival ($p<0.015$). HHSCT is a complex, high risk procedure, where numerous parameters contribute to morbidity and mortality. This might explain the different impact of IA between the HHSCT and control group, which undergoes a lower risk procedure. In any case, larger cohorts of patients enrolled in prospective studies might identify factors correlating with IA.

**P482**

**Clinical recovery following treatment of invasive fungal sepsis by antimycotic drugs and rHuG-CSF plus dexametasone mobilised granulocytes in autologous stem cell recipient**

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Background: The occurrence of severe bacterial and/or fungal infection is a dangerous complication of high-dose preconditioning chemotherapy due to autologous stem cell transplant (SCT). During longstanding neutropenia, patients are at high risk for life-threatening sepsis.
Aim: to assess the usefulness and protecting effect of recombinant human Granulocyte-Colony Stimulating Factor (rHuG-CSF) plus Dexametasone mobilized granulocyte transfusions (GTX) combined with antimycotic drugs in severe neutropenic patient with fungal sepsis after autologous SCT.
Patient and Methods: Male 42 years old patient was treated for lymphoblastic lymphoma at Clinical center of Serbia with Hyper-CVAD and due to high risk disease consolidated with autologous SCT in the complete remission in 2010. The number of reinfused CD34+ cells was 3.8x10E6/kgtm. Before GTX, severe neutropenia (absolute neutrophil count - ANC = 0.1x10E9/L) related to autologous SCT was persisted three weeks, complicated with fungal sepsis (Stephanoascus citerii and Candida guilliermondi) despite combined antimycotic (Voriconasol and Caspofungin), antibiotic, antiviral therapy and rHuG-CSF applying. Our own granulocyte-donor preconditioning regimen incorporated: rHuG-CSF 5 µg/kgbm for two days and Dexametasone 6 mg per os 12 h prior to apheresis. Granulocytes for supportive treatment were collected by Cobe Spectra (TerumoBCT, USA).
Results: The processed blood volume was 6250 mL, the quantity of collected cell suspension was 360 mL, and the number of harvested granulocytes was 6.2x10E10/Unit. After cell collection, cells were irradiated with 25 Gy to prevent transfusion associated GvHD, and after that applied across central venous catheter. Patient was premedicated with Methylprednisolone, Ranitidine and Loretadine. The *in vivo* recovery, that is mean increase of the granulocyte count one hour after GTX was 0.9x10E9/L (0.5x10E9/L to 1.4x10E9/L), with consecutive rising until normalization in seven following days. To prevent of CMV infection, Gancyclovir was applied with resolving fungal sepsis, significant decrease of C-reactive protein (CRP) and patient's condition complete clinical recovery.
Conclusion: The use of GTX and antimycotics can be a satisfactory and safe therapeutic approach with advantageous effects on invasive fungal sepsis in immunosuppressed neutropenic patients following SCT.

**P483**

**Before and after quality management system according to JACIE, screening of the pulmonary aspergillosis infection and catheter infection**

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The results of the screening on Heamotopoetics Stem Cell Transplante patients, showed that the Quality Management System has decreased the "catheter infection" ratio and "Pulmonary Aspergillosis Infection" ratio. We have already screened the 60 Stem Cell Transplate Patients before Quality Management System according to Jacie (Joint Accreditation Committee of Eurepean Bone Marrow Transplant) and 60 Stem Cell Transplate Patients after the Quality Management System according to Jacie.
Before Quality Management System there were 22 patients with "Pulmonary Aspergillosis Infection" in 60 patients and the ratio is % 36,6 and 11 patients with "catheter infection" in 60 patients and the ratio is % 18,3. After the Quality Management System there were 15 patients with "Pulmonary Aspergillosis Infection" in 60 patients and the ratio is % 25 and 5 patients with "catheter infection" in 60 patients and the ratio is % 8,3. With this QMS the ratio of "Pulmonary Aspergillosis Infection" decrease % 31,7 and "catheter infection" decrease % 55.
All selection distribution before QMS 25 Allogeneic and 35 Autologous and after QMS 25 Allogeneic and 35 Autologous. Also the room temperatures are stable between 24-26 C, humidity is stable at %50-60, pressure is stable + 14-16 Pascal, at the same building at the same rooms.
This screening study showed that the QMS can decrease the number of the infected patients, because of the rules and standars of hygneic patient care ,attention of all staff with the according to the rules of Jacie Standarts.

**P484**

**A single-centre experience of voriconazole therapeutic drug monitoring in the management of leukaemia/bone marrow transplant patients**

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Background: Voriconazole is frequently used in the prevention and treatment of fungal infections in immunocompromised patients. The nonlinear pharmacokinetics and wide inter- and intrapatient variability leads to unpredictable plasma concentrations. Studies report that voriconazole trough levels correlate with efficacy and toxicity and it has therefore been proposed that voriconazole therapeutic drug monitoring (TDM) is necessary to improve patient outcomes. A 6-month pilot project to evaluate voriconazole TDM was initiated at our institution. We sought to evaluate the appropriateness and ordering and sampling practices of voriconazole TDM in our patients.
Methods: All voriconazole TDM episodes performed on adults from May 1 to Nov 30, 2011 at the L/BMT Program of British Columbia were included in the study. Voriconazole was measured by UPLC using a UV detector. Steady-state voriconazole trough levels were defined as the following: Undetectable (less than 0.3 mg/L); Below therapeutic range (0.3-0.7 mg/L); Borderline therapeutic (0.8 – 0.9 mg/L); Therapeutic (1–5.5 mg/L) and Toxic (greater than 5.5 mg/L)
Results: 281 TDM levels were performed on 67 patients. 12 patients were excluded due to inappropriately drawn samples or missing information. Of the 214 resultant TDM episodes in 55 patients, voriconazole concentrations were outside of the therapeutic range in 54 (25.2%) TDM episodes: 6 (2.8%) were undetectable; 29 (13.6%) below therapeutic, 12 (5.6%) borderline therapeutic and 7 (3.3%) were toxic.
The baseline level was outside of the therapeutic range in 25/55 pts (45.5%). Of those 25, only 6 (24%) had their voriconazole dose adjusted and 19 (76%) did not. Reasons for non-action included not drawn at steady-state, not drawn appropriately, prophylaxis, desire to wait for a repeat level or no reason was given at all. Of those 6 patients where an action was taken, 2 patients became therapeutic, 1 borderline therapeutic, and 3 patients did not have follow-up levels drawn.
Conclusion: This pilot study highlights the importance of appropriate sampling and interpretation. 45.5% of patients had non-therapeutic baseline trough levels and only 6 of those patients had their dose adjusted. On the basis of these findings, we conclude that voriconazole TDM should be implemented to optimize patient outcomes and that there is a need for a standardized dosing adjustment algorithm to assist clinicians in interpreting and adjusting dosing for voriconazole trough levels.

**[P484]**

| Characteristics of Patients and Voriconazole TDM Episodes | |
|---|---|
| Patients | 55 |
| Male | 28 |
| Age.mean (range) | 50 (20-76) |
| Indication for voriconazole (prophylaxis/treatment) | 27/28 |
| Total TDM episodes evaluated | 214 |
| TDM per patient | 5 (1-19) |
| Patients with more than one TDM episode | 42 |

**P485**

**Allogeneic stem cell transplantation in patients with acute myeloid leukaemia and history of invasive aspergillosis – the role of secondary prophylaxis with voriconazole: single-centre experience**

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Background: Allogeneic stem cell transplantation (aloSCT) can improve outcome of patients (pts) with acute myeloid leukemia (AML). The part of pts with AML can suffer from invasive aspergillosis (IA) during remission induction treatment. These pts as potential candidates for aloSCT have higher risk of IA relapse and accompanying higher morbidity and mortality after aloSCT. Several published studies indicate that secondary prophylaxis with potent antifungal agents could decrease risk of IA relapse after aloSCT. To evaluate the role of secondary prophylaxis with voriconazole in pts with history of IA who undergo aloSCT for AML we retrospectively analysed outcome of such pts transplanted at our centre.
Patients and Methods: in period 1/2005-10/2011, 21 pts with AML (71% in 1.CR, 29% beyond 1.CR) and history of IA (38% with residual infiltrates) underwent aloSCT (81% redecud-intensity, 19% myeloablative aloSCT). The donor was in 29% HLA identical related, in 38% matched and in 33% mismatched unrelated. Source of stem cells was peripheral blood and the median of infused CD 34+cells was $5,2x10^6$/kg (range: $1,7\text{-}14,9x10^6$/kg). CsA and methotrexate were administered as GVHD prophylaxis. Voriconazole (200mg twice daily) was used as invasive fungal infection (IFI) prophylaxis from day -1. Other antimicrobial prophylaxis included norfloxacin and aciclovir. Evaluation and treatment of aloSCT complications and outcome was made according to established criteria and recommendation.
Results: All pts fully engrafted and achieved CR. 7 pts (33%) developed aGVHD (5% grade III-IV) and among 18 evaluable pts 10 (56%) of them developed chGVHD (50% extensive). With median follow-up 23 months (range, 2-64 months) 13 pts (62%) are alive in CR. 2 pts (10%) relapsed and died. 6 pts (28%) died due to NRM and 2 (10%) of them till day 100. The median time of voriconazole administration was 60 days (range, 21-131 days) and only in 1 patient (5%) voriconazole was stopped due to hepatic toxicity. With above mentioned follow-up only 2 pts (10%) developed IFI (1 case with IFI-related death). The estimated probabilities of 3-years EFS and OS are 52%.
Conclusion: In spite of relatively small number of analysed pts and retrospective type of analysis, our data suggest and also support results of previous published studies that secondary prophylaxis with voriconazole in pts with AML and history of IA undergoing aloSCT is well tolerated and can protect these pts from higher risk of IA relapse.

**P486**

**Budget impact model of voriconazole for prophylaxis of invasive fungal infections in Spanish patients undergoing allogeneic haematopoietic cell transplantation**

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Objectives: Antifungal prophylaxis (AFP) is used in allogeneic hematopoietic cell transplant (alloHCT) recipients to prevent the significant morbidity and mortality associated with invasive fungal infections (IFI). Currently, there is no standard prophylactic agent in Spain for alloHCT recipients post-engraftment. Voriconazole (VOR) is approved for the treatment of various types of IFI, with potential for an expanded indication that includes its use as primary AFP in alloHCT recipients post-transplant. The aim of this study was, therefore, to inform health care decision-makers of the possible financial implications to the Spanish national health system should VOR be approved for this expanded indication.
Methods: A budget-impact model (BIM) was constructed based on a population of 709 alloHCT patients, all of whom would be expected to receive AFP, as confirmed by expert opinion. The model considered costs from day +1 to day +100 associated with drug acquisition (excluding value added tax), switching to other licensed antifungal treatment (OLAT) due to failure or toxicity of original prophylaxis, IFI monitoring, and treating breakthrough IFI. The baseline cost model was based upon current prescribing patterns for orally available, mould-active AFP: 50% for itraconazole, 20% for posaconazole, and 30% for VOR. Three scenarios were developed, assuming a 10%, 15%, and 20% increase in VOR-based AFP, respectively. All estimates for treatment duration, drug usage, and costs were confirmed by expert opinion.
Results: Based on an average incremental cost for all 3 scenarios, the expanded indication for VOR resulted in an average cost increase of €206,323 in the population of interest (Table). This increase was derived from a baseline total cost of €10,441,526. When considering drug costs only (ie, for both prophylactic agent and OLAT), the incremental cost per patient ranged from €365 to €730 and the total incremental cost from €258,720 to €517,439. When additionally considering costs for treatment of breakthrough IFI, the incremental cost per treated patient ranged from €194 to €388 and the total incremental cost from €137,549 to €275,098.
Conclusion: Along with the assumed increase in VOR use in this setting, expanding the indication of VOR to include AFP in alloHCT recipients post-transplant was projected to minimally increase the overall cost incurred by the Spanish health system.

**[P486]**

| | Incremental cost impact of expanded indication for voriconazole* (€) | | | Average incremental impact (€) |
|---|---|---|---|---|
| | Scenario 1: 10% increase | Scenario 2: 15% increase | Scenario 3: 20% increase | |
| **Total incremental costs** | | | | |
| Prophylaxis drug costs | 301,932 | 452,898 | 603,864 | 452,898 |
| OLAT drug costs | -43,212 | -64,818 | -86,424 | -64,818 |
| *Subtotal* | *258,720* | *388,079* | *517,439* | *388,079* |
| Cost of treating breakthrough IFI | -121,171 | -181,756 | -242,342 | -181,756 |
| **Total** | **137,549** | **206,323** | **275,098** | **206,323** |
| **Per patient incremental costs** | | | | |
| Prophylaxis drug costs | 426 | 639 | 852 | 639 |
| OLAT drug costs | -61 | -91 | -122 | -91 |
| *Subtotal* | *365* | *547* | *730* | *547* |
| Cost of treating breakthrough IFI | -171 | -256 | -342 | -256 |
| **Total** | **194** | **291** | **388** | **291** |

*IFI monitoring costs are also considered in the model, but are not included in the table, because they do not change based on prescribing patterns (i.e. the incremental cost is zero).

**P487**

**Safety and efficacy of two regimens of high-dose liposomal amphotericin B for prophylaxis of invasive fungal infection in allogeneic haematopoietic stem cell transplantation: a comparative retrospective single-centre study**

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AmBisome (amphotericin B liposomal complex, L-Amb) is highly effective for the treatment of invasive fungal infections (IFI) and may be an important prophylactic drug in patients undergoing hematopoietic stem cell transplant (HSCT) specially in patients with liver impairment. We retrospectively analysed the efficacy and safety of two high dose L-Amb regimens, namely 7 mg/kg given once a week *vs.* 10 mg/kg given every 10 days, for prophylaxis of IFI in patients who underwent a HSCT at our institution between January 2005 and June 2011.
Twenty patients (median age 41 years, range 19-67) received 10 mg/kg every 10 days for a median of 5.6 weeks (range 2-25 weeks) and 13 patients (median age 45 years, range 28-65) received the weekly 7 mg/kg regimen for a median of 8 weeks (range 4-25 weeks). Indications for prophylaxis included prophylaxis in patients having GVHD treatment (n=17), secondary prophylaxis for prior IFI (n=10) and intolerance or allergy to azoles (n=6). There was no significant difference in baseline characteristics or indications for prophylaxis in the 2 groups.
Results: Both regimens were well tolerated and there were no cases of discontinuation of prophylactic treatment due to adverse events. Transient rises in serum creatinine and serum potassium levels were noted in 3 patients in the 10 mg/kg every 10 days group and in 2 patients in the weekly 7 mg/kg group. The renal toxicity was reversible in all and responded to intravenous fluids. Liver function tests remained stable in all patients analysed. We found no significant differences in the rates of breakthrough fungal infections in the 2 groups. Two patients in the 10 mg/kg every 10 days group and 1 in the weekly 7 mg/kg group developed breakthrough fungal infections.
Conclusion: We conclude that high dose L-Amb may provide useful protection against invasive fungal infections specially in patients with liver impairment and others intolerant to azoles. Both regimens of 10 mg/kg every 10 days or 7 mg/kg weekly can be administered to patients safely with few adverse events.

**P488**

**Anidulafungin primary antifungal prophylaxis in 36 high-risk haematological patients undergoing haematopoietic stem cell transplantation**

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Background: Invasive fungal infections (IFIs) constitute a substantial source of morbidity and mortality among patients (pts) undergoing allogeneic hematopoietic stem cell transplantation (allo-SCT). Anidulafungin (ANI) is an echinocandin that inhibits glucan sinthase, an important enzyme in the formation of fungal cell wall; it has a broad antifungal spectrum of action, low toxicity profile without relevant drug interactions.
Aim: ANI safety and efficacy as antifungal prophylaxis agent was tested in pts with high risk hematological malignancies receiving alloHSCT from July 2009 to March 2011.
Materials and Methods: In our institution, we analyzed 36 pts with high risk hematological malignancies (18 acute leukemia, 2 chronic myeloid leukemia, 2 non Hodgkin limphoma, 2 Hodgkin limphoma, 2 myelodysplastic syndrome, 2 myelofibrosis) undergoing allo-SCT: 19 Haploidentical SCT (Haplo), 9 Matched Related Donor (MRD), 7 Matched Unrelated Donor (MUD), 1 Cord Blood (CB). Disease status at SCT was intermediate/advanced in 21/36 pts; anti-thymocyte globulin was administered to 21/36 pts and 2/36 pts performed a previous allo-SCT. The median time from diagnosis to allo-SCT was 664 days (range: 51-4022).
Antifungal prophylaxis with ANI was started 1 day before conditioning (200 mg die iv single dose, then 100 mg die iv) until

neutrophil engraftment (PMN >0.5 x 10e9/l for 3 consecutive days) and subsequently replaced with voriconazole. Prophylaxis was primary in 34/36 pts and secondary in 2/36 pts.
Results: We observed in 1/36 pts an allergic grade II skin toxicity after the first ANI administration that was immediately interrupted. Median duration of ANI therapy was 23 days (range: 1- 44). Median time to neutrophil engraftment was 20 days (range: 12-52). 19/36 pts stopped ANI at neutrophil engraftment, without signs of IFIs. 16/36 pts stopped ANI for proven (3/19 pts) or probable IFIs (3/19 pts) (EORTC 2008 criteria) and we replaced it with Voriconazole. In the MRD setting, 7/9 pts stopped ANI for engraftment, 1 for allergic reaction and 1 for possible IFI. In the Haplo setting, 7/19 pts stopped ANI for engraftment and 5/19 for proven/probable IFIs. In the MUD setting, 5/7 pts stopped ANI for engraftment and 2/7 for possible IFI. Patient receiving CB stopped ANI for proven IFI. Overall fungi isolated in proven IFI were aspergillus.
Conclusions: ANI is a well tolerated antifungal agent and a choice as primary prophylaxis in high risk pts receiving allo-SCT.

**P489**

**Micafungin as prophylaxis in recipients of allogeneic HSCT: results of different dose levels**

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Micafungin, a novel echinocandin, has been approved for prophylaxis of candida infections in patients undergoing allogeneic haematopoietic stem cell transplantation (HSCT). However, data on micafungin's use in clinical practice are limited.
Therefore, we retrospectively determined efficacy and toxicity of primary antifungal prophylaxis with micafungin in different dosage regimens in a total of 150 adult pts undergoing allo-HSCT. Fifty consecutive patients each received micafungin at a dose of 50 mg (1/2010 to 7/2010), 100 mg (9/2010 to 4/2011) or 150 mg (5/2011 to 11/2011) once daily starting from day 0 or earlier, whether leucocyte count was ≤1000/µl.
There were no significant differences regarding age, gender, days of leukopenia, or duration of micafungin prophylaxis (16.0 *vs.* 15.4 *vs.* 16.0 days) across the 3 groups.
In those pts who received micafungin for at least 6 days, 12/46 (26%), 6/44 (14%) and 9/46 (20%) pts received empiric antifungal treatment according to local standards. However, the frequency of invasive fungal infections according to EORTC criteria did not differ significantly (7/46; 15% *vs.* 5/44; 11% *vs.* 5/46; 11%) across the different dosage groups. In the 50-mg-group there was one case of candidaemia with C. parapsilosis after 12 days of micafungin.
In all three groups, micafungin prophylaxis was well tolerated without any case of toxicity-related treatment discontinuation. Renal function was not significantly altered (median change of serum creatinine from 0.8 to 1.0 mg/dl after 9 days in each group). In 61% of pts total bilirubin significantly increased due to ATG application. In the remaining 57 pts, total bilirubin increased by median 0.8 mg/dl (baseline: 0.7 mg/dl) 6 days from start of micafungin.
The incidence of invasive fungal infections is similar irrespective of the micafungin dosage. Toxicity and tolerability of micafungin prophylaxis are good in the allotansplant setting.

**P490**

**Antigungal prophylaxis with micafungin in allogeneic stem cell transplantation, comparative results with intravenous itraconazole: a single-centre experience**

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Invasive fungal infections (IFIs) are an important cause of morbidity and mortality in allogeneic hematopoietic stem cell transplantation (HSCT). In patients with cancer a meta-analysis showed that primary antifungal prophylaxis (PAP) with mold-active antifungals agents reduced the documented aspergillosis (Robenshtok JCO 2007).
Micafungin, an echinocandin with activity against Candida and Aspergillus, has a good safety profile, even in patients with liver or kidney impairments, and it can be used at the same time with the conditioning treatment (CT). Moreover it has no significant interactions with other drugs. In the last three years (September-08/September-11) we have progressively changed our PAP with intravenous itraconazole by micafungin during the neutropenic period in HSCT. We used intravenous itraconazole on the day +1 and micafungin at the beginning of CT. Both were used until resolved mucositis and oral fungal prophylaxis with posaconazole could be started. Moreover intravenous PAP was changed by empirical antifungal treatment or pre-emptive / targeted treatment when they were needed. We present the characteristic and comparative results of the patients treated with each of these drugs, Table 1, cohort 1(itraconazole 200 mg/day, load dose 400 mg one day) and cohort 2 (micafungin 50 mg/day)
Results: Both cohorts presented the same median age (43 *vs.* 42). In cohort 2 there were more patients diagnosed with AML/MDS and HD and less with ALL and MM than in the cohort 1.
In the cohort 2 more haploidentical donors and less cord blood cells as a source of progenitors cells were used. The number of match-related and unrelated donors was similar. There were no differences in CT (MAC and RIC) and in neutrophils engraftment in both cohorts (+16). Although empiric antifungal treatment was used more frequently in cohort 1 this difference was not statistically different. (50% cohort 1 *vs.* 33% cohort 2, p:0.17).
In the neutropenic period our patients had a very low number of IFIs (two possible in both cohorts and one probable in cohort 1). No toxicity due to micafungin was observed. In conclusion during the neutropenic period in HSCT micafungin was well tolerated with no associated toxicity or drug-drug interactions. Patients receiving micafungin needed less empirical antifungal drugs compared to patients receiving itraconazole, and no developed IFI. An analysis cost-effective is ongoing comparing both drugs.

[P490]

**Table1 Baseline cohort s characteristic and results observed**

| | ITRACONAZOLE iv (40) Cohort1 | MICAFUNGIN (39) Cohort2 |
|---|---|---|
| **Period of time** | **October 06 - July 10** | **Mars 09 - September 11** |
| **Median age** | **43 (18-69)** | **42 (16-68)** |
| **Diagnostics** | | |
| AML / MDS | 17 (42.5%) | 22 (56%) |
| ALL | 9 (22.5%) | 4 (10%) |
| AA/MM /IM | 1/5/0 | 1 / 1 /2 |
| NHL/CLL / HD | 4/ 1 / 2 | 2/ 1 / 6 |
| **Source of progenitor cells** | | |
| **MRD/ MMRD** | **20 (47.5%) / 1 (2.5%)** | **14 (36%) / 0** |
| **MURD** | **11 (27.5%)** | **13 (33%)** |
| **CBT** | **7 (17.5%)** | **4 (10%)** |
| **HAPLOIDENTICAL** | **2 (5%)** | **8 (21%)** |
| **Conditioning Treat. MAC / RIC** | **25 (62.5%) / 15 (37.5%)** | **23 (59%) / 16(41%)** |
| **Neutrophils engraftment (day)** | **+16 (11-27)** | **+16 (10-29)** |
| **Empirical Treat.** | **20 (50%)** | **13 (33%)** |
| **Casp /LAMB/Vor** | **17/2/1** | **7/5/1** |
| **IFI diagnosed** | **2 possible 1 probable** | **2 possible** |

AML/MDS, acute myeloid leukemia / myelodisplastic syndrome. ALL, acute lymphoblastic leukemia. AA , aplastic anemia. MM, multiple mieloma. IM, idiopathic myelofibrosis. NHL, non Hodgkin lymphoma. CLL, chronic lymphocytic leukemia. HD, Hodgkin disease. MRD , Match related donor. MMRD Mismatch related donor. MURD Match unrelated donor. CBT cord blood transplant MAC myeloablative conditioning treatment. RIC reduced intensity conditioning treatment.
Casp Caspofungin. LAMB lipid amphotericin B. Vor Voriconazole

**P491**

**Antifungal prophylaxis with micafungin in recipients of allogeneic stem cell is effective: results from a single centre**

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Invasive fungal infections (IFI) constitute a major cause of mortality and morbidity among allogeneic hematopoietic stem cell transplant (ASCT) recipients. Because early diagnosis and treatment of IFI are the key factors for improving the prognosis, a prophylactic antifungal strategy has been recommended for high risk patients.

Objectives: A retrospective study was conducted to evaluate the efficacy and safety of micafungin antifungal prophylaxis for IFI in pediatric and adult ASCT recipients during the neutropenic phase of high risk transplants (defined as myeloablative conditioning, mismatch transplants or umbilical cord blood transplant) and in patients with graft versus host disease (GvHD) to whom posaconazole could not be administered because of oral route unavailability or gastrointestinal dysfunction.

The primary endpoint was treatment success, which was defined as no change in antifungal therapy for any reason. The secondary endpoints were the incidence of proven, probable or possible IFI, safety, and overall survival 4 and 12 weeks post-micafungin initiation.

Methods: The files of all the institution's patients (pts) who received micafungin prophylaxis between October 2009 and December 2011 were retrospectively reviewed. The clinical signs, blood culture results, serum Aspergillus galactomannan antigen results, computed tomography scans, liver function test results and adverse events were recorded and analyzed.

Results: Forty pts (29 adults and 11 children) received prophylactic micafungin at a dosage of 50 mg daily for adults and 1 mg/kg for children weighting <50 kg for a mean treatment duration of 21 days. Prophylaxis was given either during the neutropenic phase of ASCT (n=30) or to pts with GvHD (n=11). The overall success rate was 46% (45% for GvHD, 47% for neutropenic phase of ASCT). For 22 pts, antifungal prophylaxis was discontinued for the following reasons: persistant fever, which was treated empirically, in 13 pts (32%); possible or probable invasive aspergillosis in 5 pts (12%), invasive aspergillosis in 3 pts (7%) and candidemia in 1 patient (2%) (EORTC criteria). In the group of 30 pts treated during neutropenic phase, possible, probable or proved IFI were observed in only 3 pts. No toxicity or other serious adverse event resulted in treatment discontinuation.

Conclusion: Micafungin may constitute a valuable prophylactic alternative in high-risk ASCT during neutropenic phase.

**P492**

**Micafungin as prophylaxis of invasive fungal infection in patients undergoing haematopoietic stem cell transplantation**

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Background: Invasive fungal infection (IFI) such as candidiasis and mold infections cause significant morbidity and mortality in hematopoietic stem cell transplantation (HSCT). Although prophylactic antifungal therapy with fluconazole has become the standard care for these patients, it has been associated with the emergence of fluconazole-resistant Candida infections. Additionally, fluconazole is not reliably effective against invasive aspergillosis.

Methods: Between January 2010 and September 2011, We conducted a prospective study to evaluate the usefulness of the administration of micafungin (Mycamine®), a class of echinocandin, as a prophylactic antifungal therapy for patients undergoing HSCT. Micafungin was started at a daily dose of 50 mg once a day intravenously over 1 hour from day 1 after HSCT. Therapy was continued until 3 days after hematological engraftment (defined as an absolute neutrophil count of over 500/uL after the nadir).

Prophylactic success was defined as the absence of proven, probable, or suspected systemic fungal infection through the end of prophylaxis therapy and as the absence of a proven or probable systemic fungal infection through the end of the 4-week post treatment period.

Results: A total of 35 patients who underwent HSCT were enrolled in the study. Underlying diseases included acute leukemia (n = 18), myelodysplastic syndrome (n = 5), aplastic anemia (n=4), non-Hodgkin's lymphoma (n = 3), and others (n = 5). HSCT were HLA-matched sibling (n=11), matched unrelated (n=15), mismatched unrelated (n=2) or autologous (n=7).

The median durations of administration of micafungin were 14 days (range 12-17 days). Prophylactic success was achieved in 34 (97.1%) of the 35 evaluated patients. No patients showed proven or probable IFI. Micafungin was well tolerated, and none of the patients required dose reduction due to adverse effects.

Conclusions: Our results indicate the effectiveness and safety of micafungin a daily dose of 50 mg as an prophylactic antifungal therapy in patients undergoing HSCT.

**P493**

**Micafungin as primary antifungal prophylaxis after allogeneic haematopoietic stem cell transplantation – a single-centre experience**

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Introduction: Fungal infections are known as a cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (alloHSCT). Therefore effective antifungal prophylaxis is necessary. The echinocandine Micafungin is approved for the prophylaxis of candidiasis in HSCT recipients. Here we report our experience with Micafungin as antifungal prophylaxis in the setting of alloHSCT.

Patients and Methods: We retrospectively analyzed five male and two female patients (pts.) who underwent alloHSCT between december 2010 and may 2011 due to AML (n=4), MDS (n=1), ALL (n=1) and NHL (n=1), respectively. The pts. with a median age of 57 years (range 27 to 69 years) received 50 mg (n=6) or 100 mg (n=1) of Micafungin once daily as primary antifungal prophylaxis after alloHSCT. Median time of Micafungin-administration was 17 days (range 12 to 33 days), beginning between day -21 and day 0 prior to alloHSCT. In five of seven patients Micafungin-prophylaxis was switched to other drugs (predominantly Posaconazole) as breakthrough-infections or major side-effects were observed.

Results: The infusion of Micafungin was well tolerated. One patient developed an allergic drug rash and an elevation of liver enzymes, both resolving after discontinuitation of Micafungin. Three of seven pts. developed an oropharyngeal fungal colonisation caused by candida albicans occurring on day 8, 12 and 15 after beginning of antifungal prophylaxis. In addition we observed one severe breakthrough-infection with candida albicans recovered from the bloodstream on day 16 after beginning of Micafungin-prophylaxis. The pt. died from fulminant septicaemia with multi-organ failure on day +17 after alloHSCT. In the whole cohort there was no evidence of fungal-infections caused by aspergillus spp.

Conclusion: Despite its known activity against candida spp., Micafungin administered at a dose of 50 mg daily showed no sufficient antifungal activity, even against non-resistant candida-strains. Additionally, significant side-effects were observed in our cohort of patients. Therefore, the role of Micafungin as antifungal prophylaxis after alloHSCT needs to be further evaluated.

**P494**

**Clostridium difficile infection following haematopoietic stem cell transplantation in children**

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Background: Clostridium difficile infection (CDI) is one of the most common bacterial causes of nosocomial diarrhea. It is a cause of significant morbidity and mortality among hospitalized patients. There is limited information on its prevalence and outcome among pediatric patients receiving hematopoietic stem-cell transplantation (HSCT). However, some studies have reported the incidence to be as high as 20%. We aimed to evaluate the prevalence, recurrence rate and outcomes associated with CDI in pediatric patients undergoing HSCT.

Methods: All pediatric patients who underwent HSCT at The Hospital for Sick Children, Toronto, Canada, between 2001 and 2009 have been reviewed from time of admission to HSCT and up to six months post HSCT. The laboratory diagnosis of CDI was based on cytotoxin detection in Vero cells from fecal specimens for all patients with clinical signs of diarrhea. Outbreak was defined as more than 2 episodes in different patients over a period of 2 weeks. CDI recurrence and patient's outcome was documented.

Results: Between 2001-2009 a total of 797 HSCTs were performed: 431 allogeneic and 366 autologous. Median age at the time of transplant was 8.0 years (range, 1.0-19.0). We observed 83 episodes of CDI (9.8%) in HSCT recipients. 4.9% of the allogeneic and 9.3% of the autologous patients. CDI episodes occurred at a median of 4 days post-HSCT (range: day -8 to day +180) following HSCT. 78 patients responded to therapy with metronidazole and 2 patients required adding second drug- vancomycin, 3 patients didn't receive therapy. Recurrent CDI occurred in seven patients (8.3%). Only one patient developed severe CDI with pneumatosis intestinalis. The cause of death for the patient was relapse of the primary disease and adenovirus pneumonitis. None of our patients succumbed to CDI. 14 patients were diagnosed with viral gastroenteritis: 11 torovirus, 2 rota virus and 1 adenovirus. We observed 9 episodes of outbreaks.

Conclusions: CDI occurred in nearly 10% of pediatric patients undergoing HSCT, especially in autologous HSCT. Nearly all patients experience mild CDI with adequate response to antibiotic therapy. This study is the largest to date to evaluate the outcome of CDI in Pediatric HSCT recipients.

**P495**

**Incidence and susceptibility of bacterial isolates from allogeneic haemopoietic stem cell transplantation recipients: single-centre experience**

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Objectives: To examine shifts in the etiology and susceptibility of bacterial isolates from allogeneic hemopoietic stem cell transplantation (allo-HSCT) recipients.
Patients and Methods: Between January 2008 and September 2011, all positive cultures from 155 pediatric and adolescent patients undergoing allo-HSCT were reviewed.
Results: The most common pathogens were S.epidermidis (14%), Enterococci spp. (17%), Kl.pneumoniae (16%), E.coli (8%), Enterobacter spp. (7%), Acinetobacter spp. (5,5%), and Ps.aeruginosa (6,5%). Of the 873 isolates, 394 (45%) were gram-positive (GPB) bacteria and 55% were gram-negative (GNB). In the 2008-2009 period GPB accounted for 41% of all bacterial infections, whereas in the 2010-2011 period the percentage had increased to 48%. All patients received as prophylaxis ciprofloxacin and 53% of all isolates was resistant to this fluoroquinolone, and resistance increased from 38% to 65%. There was trend of increasing rates among vancomycin resistant enterococci (VRE) 4% *vs* 9,5%. S.aureus were resistant to oxacillin in 17%. We observed increasing rates of multiresistant strains of Ps.aeruginosa, Kl.pneumoniae and Acinetobacter spp. In 10% Ps.aeruginosa isolates were panresistant. Analysis of susceptibility shows high efficacy of vancomycin, linezolide and piperacillin/tazobactam, moderate efficacy of carbapenems, cefoperasone/sulbactam, quinolones and aminoglycosides, and increasing resistance to cefalosporines of III generation.
Conclusions: There is a slight prevalence of GNB and the trend of increasing role of GPB. We observed increasing rates of multiresistant strains GNB. The recommendation of cefalosporines of III generation may not be appopriate.
Keywords: bacterial infections, allogeneic hematopoietic stem cell transplantation, antibiotic resistance.

**P496**

**Outcome of non-fermentative Gram-negative rods bacteraemia in children with haematological malignancies and stem cell transplantation: 10-year experience**

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Introduction: Non-fermentative gram negative rods (NFGNR), as Pseudomonas aeruginosa (PA), Acinetobacter baumanii (AB), Stenotrophomonas maltophilia (SM) are important causes of blood stream infections in children with hemato-oncological diseases and hematopoietic stem cell transplant (HSCT). They are frequently resistant to multiple antibiotic classes.
Aim: To analyze resistance pattern of NFGNR in children with hematological malignancies and HSCT and to determine if bacteremia with NFGNR is associated with high mortality.
Methods: All episodes of NFGNR bacteremia that occurred during 2001–2011 in children with hematological malignancies or post-HSCT in our tertiary hospital were retrospectively analyzed. Pseudomonas and Acinetobacter spp. that exhibited resistance to three or more antibiotic classes, as well as all SM were defined multidrug resistant (MDR). Mortality at 7 and 30 days after infection was recorded. Two or more positive blood cultures were considered positive for Acinetobacter lwofii and Pseudomonas stutzeri.
Results: 66 NFGNR were isolated from blood cultures of 52 children, 33 male, 7 months to 17, median 5, years old. Underlying diseases included: hematological malignancy (n=40), HSCT (n=26; 22 allogeneic, 3 autologous and 1 cord blood) for malignancy/premalignancy (n=16) or nonmalignant conditions (n=10). 29 (44%) episodes occurred during neutropenia, 17 (26%) were nosocomial. In HSCT patients they occurred 0-600 (mean 159, median 57) days after transplantation. NFGNR included PA (n=30;46%), SM (13;19%), AB (7;11%); Pseudomonas spp (6;9%), Acinetobacter spp (10;15%). Mortality at 7 (attribute mortality) and 30 days after infection was 1/66 (1.5%) and 2/66 (3%), respectively. NFGNR were non-susceptible to: ciprofloxacin 6/60 (10%), carbapenems 14/66 (21%). All SM were susceptible to trimethoprim-sulfametoxazole; all 11 Acinetobacter spp. to minocycline. 1/10 (10%) of Acinetobacter spp. were non-susceptible to ampicillin-sulbactam. Pseudomonas and Acinetobacter spp. were non-susceptible to: piperacillin 8/50 (16%), ceftazidime and piperacillin-tazobactam 6/52 (11.5%) each, gentamicin 4/52 (8%), amikacin 1/52 (2%). 20% of all NFGNR were MDR, including 3/36 (8%) Pseudomonas, 3/17 (18%) Acinetobacter spp. and all 13 SM.
Conclusions: Mortality rate due to NFGNR is relatively low despite of emerging resistance. NFGNR bacteremia can frequently occur without association with neutropenia and hospitalization.

**P497**

**Empiric piperacillin-tazobactam or meropenem as first-line monotherapy in two consecutive years. Retrospective analysis of 236 bone marrow transplants (BMT) adult recipients**

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Empirical beta-lactam monotherapy has become the standard first line therapy in febrile neutropenia.
Objective: Evaluate which regimen was preferable empiric antibiotic monotherapy in BMT patients (pts).
Methods: We retrospectively analyzed 236 pts as intention to treat piperacillin-tazobactam (4x4.5 g IV/day) from May 2009 to April 2010 and meropenem (3x1 g IV/day) from May 2010 to April 2011 for BMT procedures.
We evaluated the pts for evidence of bacterial infection and glycopeptide addiction using the EORTC criteria.
Results: Fifty one pts had no fever (27 piperacillin-tazobactam, 24 meropenem). One hundred and eighty five episodes were assessable (82 piperacillin-tazobactam, 103 meropenem). Median duration of treatment was: 6 days (0-19 days) piperacillin-tazobactam 7 days (1-19 days) meropenem. The classification (EORTC) was: microbiological documentation (12.9% *vs* 23.2%), clinical documentation (15.1% *vs* 10.7%), with no explanation (60.2% *vs* 55.4%), no infectious (1.1% *vs* 0.9%). We observed similar success rates without modification (37.6% *vs* 42.5%), success with modification (1.1% *vs* 1.8%) and treatment failure (48.4% *vs* 46%). There were no differences in overall mortality rate and overall survival.
Conclusion: The result of the empirical regimen of piperacillin-tazobactam was similar to meropenem as first line monotherapy in two consecutive years in BMT recipients.

**P498**

**Safety of tacrolimus administration with aminoglycosides or glycopeptides after allogeneic haematopoietic stem cell transplantation**

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Introduction: Calcineurin inhibitors such as tacrolimus and cyclosporine A have been widely used for the prophylaxis and treatment of GVHD after allogeneic hematopoietic stem cell transplantation (HSCT) for hematological diseases. Nephrotoxicity is one of their most important toxicities. Recipients of allogeneic HSCT are highly immunocompromised and susceptible

to infectious complications, and are often treated with nephrotoxic antimicrobial agents such as aminoglycoside (AG) and glycopeptide (GP). Since nephrotoxicity of tacrolimus concomitantly administered with nephrotoxic antimicrobial agents has yet to be fully, we retrospectively evaluated its safety in the recipients of allogeneic HSCT.
Patients and Methods: Recipients of allogeneic HSCT who received intravenous AGs or GPs during the continuous intravenous infusion of tacrolimus within 30 days after transplantation were included in this study. Fifty patients fulfilled the criteria and could be evaluated. The data including patient characteristics, whole blood concentration of tacrolimus, the dose and duration of AG/GP treatment, and serum creatinine were obtained from data base and medical records. Therapeutic drug monitorings of AGs, GPs, and tacrolimus were systematically performed in all the patients.
Results: Median age of the 50 patients was 47.5 years (range: 18-60). Of these patients, there were 40 episodes of tacrolimus administration with AGs (amikacin, gentamicin, arbekacin) and 38 with GPs (teicoplanin, vancomycin). Median duration of the concomitant administration with tacrolimus was 8 days (range: 2-22) for AGs and 11.5 days (range: 4-40) for GPs. Mean blood concentrations (±SD) of tacrolimus during AG and GP administration were 17.1±2.1 and 16.2±1.6 mcg/ml, respectively. Twice or greater increases of serum creatinine compared with that before initiating AGs or GPs were observed only in 2 of 40 (5.0%) episodes with AGs and 1 of 38 (2.8%) with GPs. Nephrotoxicity observed in these patients was reversible and hemodialysis was not required.
Conclusion: Since incidence of clinically significant nephrotoxicity was low, it was suggested that tacrolimus can safely be administered concomitantly with AGs or GPs even in the early post-transplant period.

**P499**

**Neutropenic fever and the profile of bacterial infections in an early course after allogeneic stem cell transplant – a single-centre experience**

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Infectious complications after allogeneic stem cell transplant (SCT) contributes to 20-30% of transplant related mortality. In most cases of neutropenic fever any causative pathogen cannot be found. The aim of our study was to assume the frequency of the fever of unknown origin and the profile of detected bacteria, then estimate the efficacy of empirical antimicrobial therapy after SCT in our centre.
In the years 2005 -2011, in our centre 65 allogeneic SCTs were performed (30 HLA-sibling and 38 alternative donors) in 44 boys and 21 girls, in the median age of 11 years (range: 1 month-21 years). The main indications for transplant were acute lymphoblastic leucaemia (29), severe inborn immunodefficiencies (13), acute myeloblastic leucaemia (7) and severe aplastic anaemia (7). We assessed cases of fever and the profile of microbiologically proved infections in post transplant course, and the response to empiric and adjusted antimicrobial therapy.
Post-transplant aplastic phase lasted 19 days (SD=4 days). Neutropenic fever occurred in 51 patients. In 16 children, the clinical state (sepsis, diarrhoea, balanitis, cystitis or toxic epidermolysis) and microbial findings were found a cause of fever. In last 35 patients we diagnosed fever of unknown origin (FUO). First line treatment (piperacillin/tazobactam + aminoglycoside) was effective in 37% patients. The most effective next line therapy in FUO was a combination of polypeptide or karbapenem and a new antifungal agent.
The profile of bacteriemia in the post transplant course (aplastic and post-engrafment phases) and adjusted therapy are shown in Table 1. Staphyloccus epidermidis (MRSE in 50%) was detected in 47% of positive blood cultures. Lower respiratory tract was infected the most frequently with Pseudomonas aeruginosa or MRSE (cultured in BAL material). Gastrointestinal tract was infected by Enterococcus faecium in 41% of positive stool cultures (HLAR in 10%), besides Enterobacter hafniae, Citrobacter freundi, Proteus mirabilis and Clostridium difficile were frequently found. In urinary tract we found mostly Enterococcus faecium and Proteus mirabilis.
Conclusions:

1. In empiric therapy in FUO teicoplanin (but not vancomycin) or karbapanem with a new generation antifungal agent were highly effective.
2. The most of cultured pathogens were sensitive only to polypeptide agents, linezolid or karbapenems.

**[P499]** **Table 1. Bacteriemia detected in post transplant period**

| Culture | Cases | Effective treatment |
|---|---|---|
| Staphylococcus epidermidis (MRSE MLS) | 8 (4) | Imipenem/Cilastatin Linezolid, Vancomycin, *Vanco+Netro+Meropenem*, Linezolid + Meropenem |
| Staphylococcus haemolyticus | 1 | Linezolid |
| Staphylococcus hominis | 1 | Meropenem + Doxicicline |
| Streptococcus oralis | 1 | Linezolid + Imipenem/Cilastatin |
| Pseudomonas aeruginosa | 2 | Imipenem/Cilastatin +Amikacin |
| Acinetobacter baumani | 1 | Imipenem/Cilastatin |
| Enterococcus faecalis | 1 | Imipenem/Cilastatin |
| Enterococcus faecium (HLAR) | 2 (1) | Linezolid |
| Stenotrophomonas maltophilia | 1 | Contaminated venal access removal |

**P500**

**Monitoring of cytomegalovirus-specific CD8+ T-cell response using QuantiFERON-CMV assay in adult allogeneic haematopoietic stem cell transplant recipients**

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Introduction: Cytomegalovirus (CMV) infection in bone marrow transplant (BMT) patients is a major cause of morbidity and mortality. Approximately 60-70% of CMV-seropositive patients experience CMV reactivation by 3 months after BMT and 20% of them experience CMV disease. CMV infection causes a strong virus-specific cytotoxic T-cell response thus the evaluation of T-cell response will be a useful tool in monitoring and predicting CMV infection in BMT patients. In this study, we monitored CMV-specific immune response in 61 adult BMT patients with QuantiFERON-CMV assay (Cellestis Ltd, Melbourne, Australia) in correlation with CMV antigenemia.
Methods: 61 patients who received allogeneic BMT (29 patients from HLA-matched sibling donor, 31 patients from HLA-matched unrelated donor and 1 from HLA-mismatched unrelated donor) were selected for this study from October 2008 to April 2011. Monitoring schedule was on week 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 after BMT, but we did not exclude those patients who could not follow the monitoring schedule due to death. Median duration of immune monitoring period was 48 weeks (range: 6-48). QuantiFERON-CMV test was performed according to the manufacturer's instructions. CMV antigenenemia assay was performed with immunofluorescence staining method.
Results: Prevalence of CMV antigenemia and positive CMV-specific CD8+ T cells on scheduled time is shown in Figure 1. 43 patients experienced positive-CMV antigenemia at least for one time. Recovery of CMV immunity was achieved faster in recipients from sibling donors (median time: 4 weeks) than those from unrelated donors (median time :7 weeks). Among 24 patients with sibling donors whose CMV quantiferon status was known, 19 patients with CMV quantiferon-positive donors showed shorter time-to-positive-CMV quantiferon results (median: 3 week) than 5 patient with CMV quantiferon-negative donors. (median 12 week). Patients with positive-Quntiferon-CMV showed less CMV infection on week 8 and10 than patients with negative (Table 1).
Conlusion: Pretransplant CMV serostatus and/or Quantiferon-CMV positivity of donor seems to affect the acquisition of CMV-specific immunity and the occurrence of CMV infection in BMT recipients. Screening of donor CMV immunity using QuantiFERON-CMV test would be useful for selection of donors in order to reduce the morbidity and mortality from CMV infection in recipients.

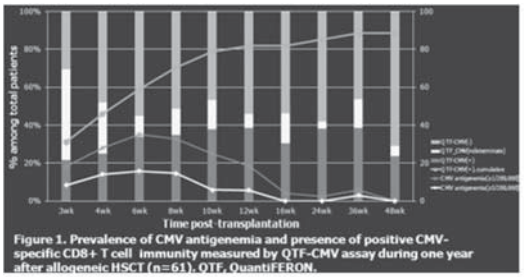

Figure 1. Prevalence of CMV antigenemia and presence of positive CMV-specific CD8+ T cell immunity measured by QTF-CMV assay during one year after allogeneic HSCT (n=61). QTF, QuantiFERON.

Table 1. Incidence of post-HSCT CMV infection in correlation with CMV- specific CD8+ T cell immunity

| | | No. of patients with CMV antigenemia(+) in accordance with QTF-CMV result, N (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 3 week (n=60) | 4 week (n=57) | 6 week (n=57) | 8 week (n=55) | 10 week (n=53) | 12 week (n=55) |
| QTF-CMV | Positive | 4/19 (21) | 6/21(29) | 10/27 (37) | 7/27 (26) | 3/28 (11) | 5/29 (17) |
| | Negative | 4/41 (10) | 7/36 (19) | 10/30 (33) | 10/28 (36) | 6/25 (24) | 3/26 (12) |

**P501**

**Cytomegalovirus viral load measurement in whole blood/ leukocytes versus plasma in allogeneic haematopoietic stem cell transplant recipients**

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Introduction: CMV disease (CMVD) has historically been a major cause of morbi-mortality after allo-HSCT. Careful monitoring and pre-emptive therapy of CMV reactivation has become standard practice in order to prevent from CMVD. For different reasons, including the frequent presence of post-HSCT leukopenia, genome detection by quantitative PCR has become the method of election for CMV monitoring in the allo-HSCT setting. Plasma is the most frequently used sample, but the measurement of the CMV load in cells from peripheral blood (PB) might estimate more accurately the viral kinetics.
Objectives: To address the sensitivity and the precocity of detection of CMV infection by qPCR in whole PB compared to plasma samples, and the correlation between CMV loads in PB and in plasma in the allo-HSCT setting. Samples and methods: during a period of 6 months (May to November 2011), 72 CMV blood samples from 15 HSCT pts with CMV infectious episodes were processed. CMV DNA was purified from 500 mcL of PB or plasma, by an automated extractor (Ampliprep-COBAS, Roche Diagnostics, Switzerland), where 500 mcL of MEM-EDTA were added before the process. A Real-Time PCR was performed with a pair of primers and a MGB probe against a fragment of CMV-glycoprotein B gene and TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems, USA) for quantification. Viral load was expressed as copies/mL (c/mL). In PB, viral load was transformed into c/$10^5$ leukocytes, according to the number of leukocytes previously counted in each sample.
Results: CMV was detected in 48 PB [10 < log 2 (< 100 c/mL), 12 log 2-3, 25 log 3-4, and 1 > log 4], and in 16 plasmas [1 < log 2, 5 log 2-3, 7 log 3-4, and 3 > log 4] ($p<0.0001$). The average viral load was 3.28±05 c/mL (2-4.58) in PB, and 3.31±0.66 c/mL (2.3-4.3) in plasma. CMV was identified at the same time in both samples in 5 episodes, earlier in PB in 4, and was only present in PB in 11. When viral load from PB was transformed into c/$10^5$ leukocytes, CMV load was: 36 < log 2, 10 log 2-3, and 2 log 3-4; the average number of c/$10^5$ leukocytes was 1.8±0.71 (0.5-3.75). A significant correlation between PB and leukocytes was found.
Conclusions: 1) CMV detection in PB was more sensitive than in plasma, which might be useful to prevent from CMVD in highly immunosuppressed pts. 2) CMV infection was detected earlier by PCR assays in PB than in plasma. 3) CMV load was similar in PB and plasma. 4) There was a correlation between viral load in PB and leukocytes.

**P502**

**Effect of steroids in CMV-specific immune response after allogeneic stem cell transplantation**

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Cytomegalovirus (CMV) infection remains a frequent complication after allogeneic hematopoietic stem cell transplantation (allo-SCT). One of the most important factors for preventing and clearing CMV infection is the development of an adequate immune reconstitution post-transplant. There is accumulating evidence that the number of lymphocytes CMV-specific (CD8/IFN-gamma) influences the risk of CMV infection and its recurrence. It's also known that graft versus host disease (GVHD) and steroid administration increase the incidence of CMV infection. However, the effect of steroid therapy on

CMV-specific immune response and its correlation with the CMV infection has not yet been studied.
Hypothesis: Quantification of CMV specific immune response and to receive or not steroids could stratify patients in different groups of risk for CMV infection.
Patients: Twenty-two consecutive patients who underwent allo-SCT from June 2010 to January 2011 in one single centre (Table 1).
Methods: Lymphocytes CD8/IFN-gamma was enumerated by flow cytometry (FC), after incubation of blood samples with pp65 and IE-1, at +30, +60, +100 and +180 days post-transplant. Global immune-reconstitution (B, NK, CD8 and CD4 lymphocytes) at days +100 and +180 was also studied. CMV viral load was monitored by quantitative PCR.
Results: In no single patient CMV-specific immune response was detected during the first 2 months after allo-SCT. Eleven

**[P502]**

| Table 1. Patients Characteristics | |
|---|---|
| ***Patients*** *(male/female)* | 22 (15/7) |
| ***Age*** *(median)* | 41 years (21-66) |
| ***Donor***<br>Unrelated<br>Sibling | 17<br>5 |
| ***Serology CMV Donor/Receptor (D/R)***<br>D-/R+<br>D+/R+/ D+/R-<br>D-/R- | 8<br>10<br>4 |
| ***Conditioning Allo-SCT***<br>Myeloablative<br>Non myeloablative | **14**<br>**8** |
| ***Source of Stem Cell***<br>Peripheral Blood<br>Bone Marrow | 19<br>3 |
| ***Prophylaxis GVHD***<br>Cyclosporine A/Metotrexate<br>Cyclosporine A/Mycophenolate<br>With **ATG** | 10<br>12<br>7 |
| ***CMV Reactivation***<br>D-/R+<br>D+/R+ | **11**<br>7<br>4 |
| ***Steroid therapy for GVHD*** | **13** |

Figure 1

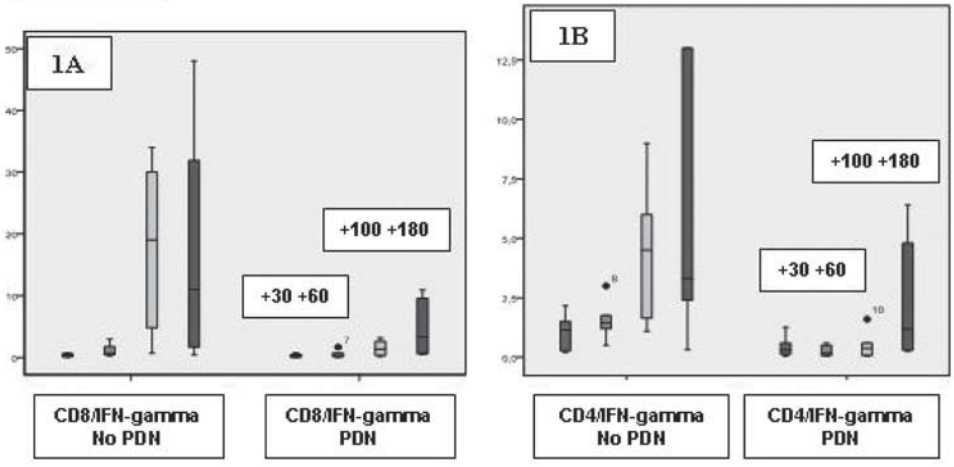


**Figure 1**. Mean levels of CD8/IFN-gamma lymphocytes (1A) and CD4/IFN-gamma lymphocytes (1B) at days +30, +60, +100 and +180. Attending to the effect of steroid therapy in the CMV-specific immune response, patients who were receiving steroids had lower CD8/CD4/IFN-gamma lymphocytes and its CMV-specific immune response occurred later (+100 days).

patients (50%) had CMV infection at a median time of +45 (38-84), and 2 of these patients developed CMV disease (1%). None of the 11 had detectable CD8/IFN-gamma lymphocytes at the time of the infection. Of the patients with CMV infection, 82% (n=9) were receiving steroids for GVHD treatment. Of these, 67% (n=6) had recurrent CMV infection and needed more days of antiviral. All patients achieved CMV-specific immune response after the first CMV infection, except those who were receiving steroids and didn't get it after the second or third infection. Patients in treatment with steroids had lower CD8/IFN-gamma lymphocytes (Figure 1). Of note, there were 30% of patients without CMV-specific immune response who didn't reactivate CMV and those patients were not receiving steroids.
Conclusions: Steroids therapy is the main risk factor for CMV infection in the post-transplant period due to its effect on delaying CMV-specific immune response. According to these results, we are designing an individualized strategy of CMV infection treatment post-allo-SCT.

**P503**

**Importance of drug-induced cytopenias in allo-HSCT patients receiving pre-emptive therapy with valganciclovir for cytomegalovirus reactivation**

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Background: Cytomegalovirus (CMV) disease has historically been a major cause of morbi-mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). During the nineties, the implementation of preemptive strategies resulted in a dramatic reduction of CMV disease. During the past decade, introduction of valganciclovir (VGCV) helped significantly improve the management of pts with CMV reactivation (CMVr). However, VGCV, as a potentially myelotoxic drug, can lead to cytopenias and their clinical consequences. The purpose of this study was to determine the effect of VGCV on peripheral blood counts in allo-HSCT pts with CMVr.
Design and Methods: We studied 54 pts who underwent consecutive allo-HSCT in 2009 and 2010. Median age was 49 years (range: 6-63). The majority (87%) received ATG as part of the conditioning at a median dose of 2.37 mg/kg (range: 1-8.75). All pts received acyclovir, but none of them anti-CMV prophylaxis. All pts received CMV seronegative and leukodepleted blood components. The pts were monitored for CMVr in whole blood and leucocytes with both quantitative PCR and pp65 antigenemia.
Results: CMVr occurred in 66.7% of cases, at a median time of day +58 post-HSCT (range: -7 to +790). Older age, ATG-containing conditioning, grade III-IV acute GvHD, and ≥2 immunosuppressive drugs were found to be risk factors for CMVr. Treatment with VGCV was given in 87.9% of cases. Induction therapy lasted 14 days (range: 3-25) and maintenance therapy 21 days (range: 14-28). After VGCV treatment, 23% of pts developed significant anemia (hemoglobin <8.5 g/dL), and 16% significant thrombocytopenia (platelets <20.000/mcL), requiring PRBC and/or platelets concentrates transfusions. On the other hand, 22.6% of pts had VGCV-related neutropenia (RAN < 500/mcL), requiring the administration of granulocyte-colony stimulating factors (G-CSF) for a median time of 5 days. In all cases, VGCV-related cytopenias were reversible.
Conclusion: CMV reactivation continues to be very common after allo-HSCT. In spite of the fact that the introduction of oral VGCV has improved dramatically the management of CMVr in this setting, a considerable of pts will develop significant cytopenias. The knowledge of this potential adverse effect of the drug is very important in order to avoid the presentation of potentially life-threatening clinical consequences.

**P504**

**Prophylactic treatment with nilotinib for post-allogeneic stem cell transplantation cytomegalovirus infection: interim analysis of a phase II trial**

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Objectives: Cytomegalovirus (CMV) infection is one of the important complications after allogeneic stem cell transplantation (allo-SCT), especially in Taiwan, where the CMV seropositive rate is more than 90% in adults. (Lu SC, Kaohsiung J Med Sci. 1999) Platelet-derived growth factor-alpha (PDGFRa) activation was reported to be critical for CMV infection. (Soroceanu L, Nature 2008) This trial aims to test whether PDGFRa inhibition by nilotinib effectively prevents patients from CMV infection after allo-SCT. The ClinicalTrial.gov identifier for this trial is NCT01252017.
Methods: Patients received nilotinib 200 mg/day after engraftment. Plasma CMV DNA copies were monitored by real-time Q-PCR at least once a week. Failure of prophylaxis was defined as plasma CMV copies higher than 10,000/ml or, regardless of levels, initiation of other anti-CMV treatments for definite or clinically suspected CMV disease. Primary endpoint was defined as rate of successful prophylaxis by day+100 after transplant. A Simon two-stage design considered the treatment unfeasible if less than nine successful patients were observed among the first 14 patients, otherwise the trial is planned to continue accrual up to a total of 31 patients.
Results: Between Dec. 2010 and Dec. 2011, 13 patients were enrolled. At the time of this analysis, 12 patients were evaluable, including four males and eight females. The median age for evaluable patients was 48.6 years. Eight patients received fludarabine and two, antithymocyte globulin, in their conditioning courses. The median time of starting nilotinib was day+20; the median duration of nilotinib treatment was 80 days. No patients had nilotinib-associated grade 3/4 adverse effects and none had early treatment discontinuation for toxicities. Prophylaxis was successful in nine patients: in four, plasma CMV was continuously undetectable; in the other five, there were subclinical, asymptomatic elevations which later resolved spontaneously. For the three failed patients whose plasma CMV copies were higher than 10,000/ml, one elected to continue nilotinib treatment; the other two switched to ganciclovir treatment. The CMV levels of all these three patients declined smoothly later and none of them developed CMV diseases during the follow-up period.
Conclusion: Nilotinib is safe and well tolerated and appears to be feasible in this population of transplanted patients. Accordingly current results permit the trial to continue to the second stage.

**P505**

**Protective effect of the treatment of Sirolimus as part of the prophilaxis of the graft-versus-host disease in haematopoyetic stem cell transplantation**

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Introduction: The Sirolimus based treatment is seen as protective against the reactivation of Citomegalovirus (CMV) infection in the context of haematopoyetic transplantation, though the evidence level is not strong enough in the international literature. In this paper we describe the acquired experience regarding this issue in our Hospital Clínico of Salamanca along the last 4 years.
Methods: 36 patients who underwent haematopoyetic transplantation have been analyzed from 2007 to 2011. It has been performed a retrospective analysis placing the patients into 2

similar groups of 18 patients each. The first cohort comprised those who underwent a Ciclosporine-Metrotexate (CM) based treatment, whilst the other one comprised the patients treated with Sirolimus(S). Donors and recipients pretransplantation serologies were taken into account as well as Polymerase Chain Reaction (PCR) for Citomegalovirus (CMV) measurements were performed until the day +100. A non-myeloablative regimen was performed on 4 patients belonging to the S regimen and on 10 patients treated with the CM based treatment.
Results: The myeloablative conditioned regimen of CM treatment was performed on the 45% of the patients, being the 55% treated with the non-myeloablative regimen. Regarding the treatment with S the ablative regimen made up the 78% of the patients and the 22% was formed for those of the reduced intensity regimen. The pretransplantation serologies against CMV on the patients treated with CM, showed Positive Inmunoglobuline G(IgG+) and Negative Inmunoglobuline M(IgM-) values in the 100% of them. In the recipients group, 2 of them (11%) had IgG- values whilst the remaining 89% had IgG+ values. All of them were IgM-.In the cohort of patients treated with S, recipients, showed IgG and IgM with the same values than the CM cohort. The donors showed IgG- in 27% of the cases and the remaining 73% were IgG+.
In the group of the patients with the CM-based treatment at the day +100, the 50% of them registered positive PCR serologies for CMV, whilst in the group of the patients treated with the S regimen, only the 16% of them registered positive PCR values.
Conclusions: In our Hospital we realized about the protective effect of the S based treatment regimen, agreeing with the literature available regarding this issue. Clinical trials are been done to clearly demonstrate this fact.

**P506**
**Cytomegalovirus meningo-encephalomyeloradiculitis after cord blood transplantation: successful treatment with systemic ganciclovir**
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Background: Meningoencephalomyeloradiculitis (MEMR) after stem cell transplantation is a very rare syndrome, characterized by extensive involvement of the nervous system at different levels, including brain, medulla and spinal roots.
Case report: We describe a 3-year male patient, 4 months after unrelated cord blood transplantation (CBT) presenting with prodromal febrile illness, followed by a wide infection of the nervous system with myelitis and less severe meningitis, encephalitis and polyradiculopathy.
Clinical symptoms: The syndrome was characterized by subacute onset of leg weakness and gait disturbance. The boy was unable to sit or stand. Bladder function was normal. The disease was diagnosed by neurological examination, serologic determinations, CMV-specific polymerase chain reaction in cerebrospinal fluid and serum, and characteristic magnetic resonance imaging scan of the spine. A neurological examination revealed the presence of myeloradiculopathy causing a significant weakness in the leg muscles. Neck stiffness and Kernig sign were also observed. Deep tendon reflexes were present in the lower extremities. A lumbar puncture yielded cerebrospinal fluid (CSF) containing normal number of neutrophils. Peripheral blood: oligoclonal IgG band. Serum CMV-DNA-emia was not detected at diagnosis, while CMV-DNA load in CSF was 2.26x10E2/mL. It was cleared after one-week therapy, with no EBV-DNA load in CSF and serum. MRI study showed two hipointensive lesions located in the cerebellum and in the periventricular area. The MRI revealed also an increased signal in lumbosacral roots. The patient was treated with systemic intravenous ganciclovir and steroids. The symptoms abate and the patient has recovered completely within 4 weeks, without neurological sequelae. This case suggests that ganciclovir with steroids appear to be effective for the treatment of CMV-induced MEMR in immunocompromised patients following CBT.
Conclusions: CMV is not known to be highly infectious for the nervous system, however in this case of MEMR, the presence of DNA sequences in the CSF suggests that CMV plays a role in the development of this newly described syndrome. Clinical, immunological and neuroimaging findings indicate that post-infectious immune mediated inflammation seems the most probable pathogenetic mechanism in this disease.

**P507**
**Cytomegalovirus ventriculitis after haematopoietic stem cell transplantation: a rare medical emergency**
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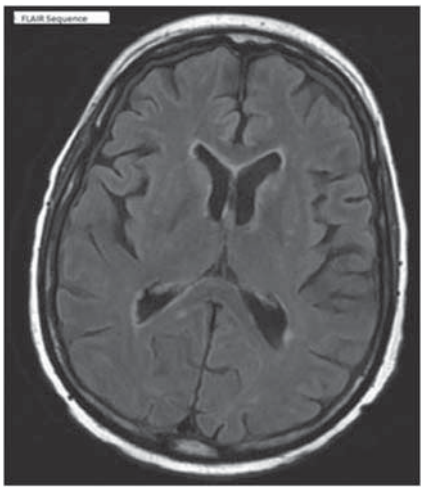
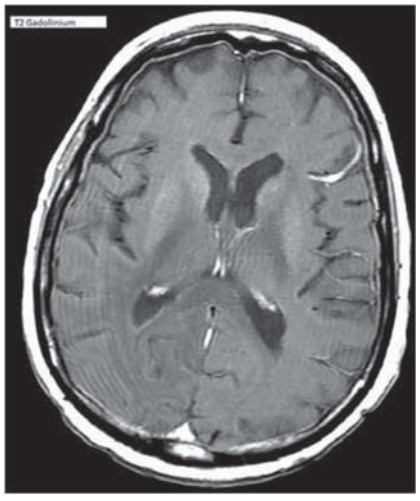
Introduction: Ventriculitis is a rare but severe localisation of CNS cytomegalovirus infection. CMV ventriculitis has a dismal prognosis thus needing a early diagnosis and has not been described to our knowledge after allogenic hematopoietic stem cell transplantation.
Clinical Case Description: We describe here a 44 years woman with an aggressive nodular pleiomorphic T-cell lymphoma.
She received several different chemotherapy regimen before having an allogenic bone marrow transplantation from unrelated compatible donor with a reduced intensity conditioning. The post-transplant period was complicated by an acute skin GVHD, then followed by an extensive chronic skin GVHD.
An asymptomatic CMV replication was detected starting from D30 post-graft. She was treated repeatedly by valganciclovir. At the 7th month after allograft, the patient was hospitalised in emergency for the following clinical symtpoms: aggravation of skin GVHD, confusion with major impaired awareness but without any neurological focal signs. She was also complaining of headache since a few days.
At admission, cerebral CT scan without iodine contrast showed no abnormality and the blood CMV PCR was negative. Initial therapy was especially acyclovir in the hypothesis of herpers simplex encephalitis. Initial attempt of lumbar puncture failed. After progressive impairment of neurological status (decrease of Glasgow Scale to 5) over 5 days, an MRI was performed and showed abnormal contrast enhancement of the ependymal lining around ventricles associated with frontal punctiform hypersignals of white matter. On the following day, the CSF analysis showed a highly positive CMV PCR and the blood CMV PCR became positive on day 7 after admission. Despite initial improvement after treatment by ganciclovir, the patient eventually died two months after onset of ventriculatis.
Discussion: We describe here a rare localisation of central nervous system CMV disease. The clinical picture is often misleading, due to the paucity of specific clinical signs. The diagnosis should be systematically questioned in severely immunodeficient HSCT recipients with a past history of cytomegalovirus pelication presenting with headache, even with a normal CT scan. Furthermore this case highlights the possible discrepancy between CNS CMV disease and blood virus load. Early MRI appears to be the investigation of choice together with the CSF analysis for this affection.
Early therapy against cytomegalovirus could lead to improvement or cure as in AIDS patients.

**[P507]**




**P508**

**Severe CMV encephalitis associated with JC virus cystitis - a rare complication after allogeneic stem cell transplantation in children**

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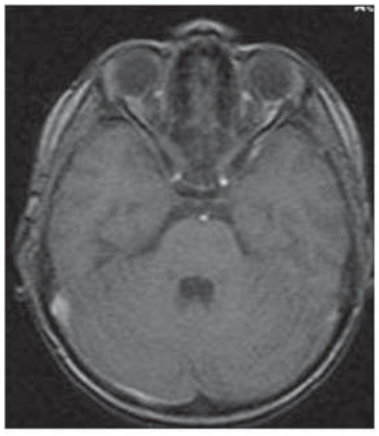
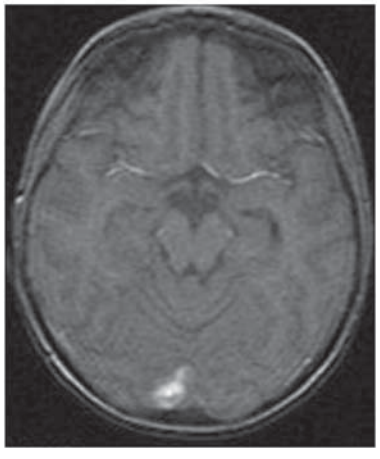
Background: Viral central nervous system (CNS) infections have been less frequently reported in patients after allogeneic stem cell transplantation (SCT) and limited data are available on their characteristics. The main etiology of viral encephalitis is HHV-6 and with lower incidence HSV, CMV, VZV, EBV, JC virus or adenovirus. The CMV encephalitis is severe and frequently is responsible for death. The polyoma JC virus associated haemorrhagic cystitis is also a severe complication after allogeneic SCT. We report a rare association of these severe complications in a young children after allogeneic SCT.
Aim: To asses the incidence and clinical manifestations of CNS viral infection in allogeneic transplant recipients in our center.
Material and Methods: We retrospectively analyzed 10 children transplanted in our center between 2003-2010. All patients received sibling HLA-matched allogeneic transplants and they were monitorized for viral infections with weekly PCR screening for CMV, EBV, HSV, VZV, JCV, BKV, HHV-6, adenovirus.
Results: We analyzed the results of 10 patients, 5M/5F, median age 5 y, range 1 -15 y, transplanted din our center for ALL – 2 cases, AML – 4 cases, SAA- 3 cases, beta thalassemia – 1 case. All patients received HLA identical sibling transplants. The infectious status before transplantation showed: 10/10 CMV D+/R+, 9/10 EBV D+/R+, and 1/10 EBV D+/R-. The viral monitoring showed 4/10 CMV reactivation, 1/10 EBV reactivation with consequent evolution to posttransplant lymphoproliferative disease. Three cases developed CMV disease: gastroenteritis- 1 case, pneumonia – 1case, hepatitis and encephalitis -1 case. 1/10 cases had a rare association of CMV encephalitis and hepatitis and JCV severe infection.
Discussion: The differential diagnosis between viral encephalitis and leukoencephalopathy is clinically difficult, but the normal values of CSA level and MRI lesions (Figures 1 & 2) associated with positive PCR tests for CMV ruled out the CSA toxicity. The patient had a CMV related encephalitis and hepatitis with favorable outcome under i.v. ganciclovir treatment associated with decreased immunosuppresion. The majority of clinical reports focused on CMV encephalitis showed an unfavorable outcome for these patients. The JC virus related hemorrhagic cystitis responded only to the decreasing of corticosteroids. The association of these 2 viral complications seems to be related to the period of treatment with high dose of corticosteroids.





**P509**

**Expression of Toll-like receptors and Betaherpesvirus reactivation in the early period after allogeneic stem cell transplantation**

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Introduction: Beta-herpesviruses, such as CMV and HHV6, are important pathogen in transplanted patients. Innate and adaptive immune response against these viruses involves the activation of Toll-like receptors (TLRs). Endosomal TLRs (TLR3, 7, 8 and 9) recognize viral nucleic acids and some surface TLRs may be involved in the detection of structural proteins. Clinical and experimental evidences indicate that CMV and HHV-6 can modulate the immune system and influence the immune reconstitution after SCT. However, the role of TLRs in this complex interplay remains unclear, especially in the setting of allogeneic SCT.
The aim of this study was to evaluate the expression of TLRs on lymphocytes and monocytes in relation to CMV and HHV6 reactivation in the early period after allogeneic SCT.
Methods: CMV and HHV6 reactivation was monitored weekly by quantitative real-time PCR. The expression of TLRs on lymphocytes and monocytes was analysed by flow cytometry as mean fluorescence intensity at day +30 and in any case before CMV or HHV6 reactivation. Functional data were obtained by ELISA assay after TLRs activation. The cell supernatants were collected and assayed for TNF-alpha, IFN-gamma and MCP-1. Relative induction of these cytokines was calculated in relation with unstimulated controls.
Results: CMV reactivation within 2 months after transplantation was observed in 13 out of 33 patients. HHV-6 reactivation was detected in 1 patient. TLRs expression and function did not significantly differ in controls and patients without CMV. Lymphocytes of patients with CMV reactivation showed an increased expression of TLR5 (4.1±2.4 *vs* 2.0±1.7 p=0,008). TLR8 expression was lower on monocytes with CMV reactivation (0.8±0.9 *vs* 2.0±1.7 p=0,03). MCP-1 relative induction post-stimulation of TLR1 and 8 was significantly decreased in patients with CMV reactivation (p<0.04).
Conclusion: Surface TLR2 and intracellular TLR3 and 9 are reported to recognize CMV by some authors. In our study, surface TLR5 and intracellular TLR8 seem to be involved in the interaction between CMV and the immune system of transplanted patients. In particular, TLR8 could play a protective role. MCP-1 production upon TLR1 and 8 activation negatively correlates with CMV reactivation. The defective immune system after SCT could explain these results, which could be confirmed by the assessment of a larger number of patients and the analysis of other possible interfering factors.

**P510**

**Reconstitution of human herpes virus 6 (HHV6)-specific T-cell immunity after paediatric HSCT**

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Human herpes virus 6 (HHV6) is known to reactivate after hematopoetic stem cell transplantation (HSCT) and has been suggested to be associated with delayed engraftment, and increased mortality. At present, little data are available on specific immune reconstitution to HHV6, considering also that viral reactivation is a very early event after HSCT, and assessment of immune reconstitution in the immediate post-transplant period may be difficult to achieve.
Aim of the present study was to prospectically analyze cellular immune reconstitution to HHV6 in pediatric recipients of allogeneic HSCT from a HLA-haploidentical family donor (haplo-HSCT) or from a matched unrelated donor (MUD-HSCT).
We analyzed the frequency of HHV6-specific IFN-g secreting cells, assessed by ELISPOT assay, in 13 recipients of haplo-HSCT and 13 recipients of MD-HSCT. HHV6 DNAemia by RT-PCR was also evaluated.
We observed that, when present, cellular immunity to HHV6 was a early event after HSCT. Indeed, the peak frequency of HHV6-specific, IFNg-producing cells was observed at 1-2 months after transplantation, in both recipients of haplo-HSCT and MUD-HSCT. In detail, the median frequency of HHV6-directed cytokine-producing cells was 5 spot forming units (SFU)/10e5 peripheral blood mononuclear cells (PBMC) (range: 0-213) in the haplo cohort, compared to 20 SFU/10e5 PBMC (range: 0-261) in the MUD-HSC group. There was no statistically significant difference in the response to HHV6 in the 2 cohorts, indicating that pediatric recipients of haplo-HSCT are able to show an efficient early response to the virus. The frequency of HHV6-directed lymphocytes was comparable to the response observed for adenovirus, with some patients showing responses in the same range as those to CMV. HHV6 reactivated in a low number of patients (4/13 haplo-HSCT and 1/13 MUD-HSCT), with a median viral DNAemia of 1050 copies/ml. The median frequency of HHV6-specific T-cells in the patients with reactivation was 1.5 SFU/10e5, compared to 6 SFU/10e5 (p=ns).
In conclusion, reconstitution of cellular immunity to HHV6 is an early event after HSCT and may confer protection also in recipients of haplo-HSCT.

**P511**

**Human herpes virus-6 reactivation after allogeneic haematopoietic stem cell transplantation**

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Background: Human herpes virus 6 (HHV-6) is increasingly recognized as a potentially life-threatening pathogen in recipients of allogeneic HSCT. Still, the actual incidence of HHV-6 infection in recipients of HSCT and the causative link between infection and clinical complications remain elusive.
Methods: From January 2009 to November 2011, we retrospectively evaluated 44 consecutive adult patients (median age 50 years) who developed positivity to HHV-6 after allogeneic HSCT for high-risk hematological malignancies and are here described. Stem cell donor were family haploidentical (33), HLA identical sibling (5), unrelated volunteer (4), cord blood (2). At the time of positivity all patients were receiving acyclovir except 4. Nineteen patients had clinical acute GvHD at time of HHV-6 positivity (grade III-IV in 13), and 36 were receiving 2-4 immunosuppressive drugs. A concomitant CMV positivity was detected in 12 patients, a severe neutropenia in 12. The viral load was determined by quantitative PCR in cell-free body fluids.
Results: Median time from allogeneic HSCT to HHV-6 reactivation was 35 days (range: 7-625). In 24 patients HHV-6 was detected in plasma, 19 had concomitant fever, 8 skin rash of new onset, 6 impaired liver function, and 5 developed cytopenia subsequently to the infection. In 6 patients HHV-6 was detected in the bone marrow, while in 9 cases, all febrile, on bronchoalveolar lavage samples. In 16 patients, HHV-6 was detected in gastrointestinal biopsies: 11 with documented gut aGvHD, 12 with diarrhoea. HHV-6 was found in cerebrospinal fluid in 5 cases (all within 30 days after HSCT), associated with high HHV-6 viral load; all these patients experienced clinical encephalitis with abnormal findings on brain MRI. HHV-6 positivity led to antiviral treatment only when associated with clinical manifestations (n=25), and first choice therapy was foscarnet: 13 (52%) completely solved the clinical event, whereas 10 (40%) died. Amongst the total 44 patients with documented HHV-6 positivity, 13 completely solved the clinical event, whereas 21 (48%) died.

Conclusions: HHV-6 reactivation is associated with distinctive clinical patterns (GI and encephalitis) in patients who undergo allogeneic HSCT. A standard monitoring of HHV-6 DNA in allogeneic HSCT recipients may be useful for identifying active infection and the introduction of a pre-emptive treatment with foscarnet.

**P512**

**Rabbit-derived ATG but not horse-derived ATG in the conditioning induces a post transplant *in vivo* imbalance between B and T cell recovery resulting in high risk of EBV-associated PTLD**

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After reduced intensity conditioning with fludarabin, busulphan and Lymphoglobulin (horse-derived ATG, hATG) followed by alemtuzumab-based T cell depleted (TCD) allogeneic stem cell transplantation (allo-SCT) in our patient cohort CMV reactivations were frequently observed, but not EBV-associated post-transplant lymphoproliferative disease (PTLD). After the enforced replacement of hATG by Thymoglobulin (rabbit-derived ATG, rATG) CMV complications remained similar but an unacceptable incidence of 26% of early EBV-PTLD was observed. In this study, we analyzed the cause of this immune escape of EBV infected B cells by measuring antibody levels and specificity in relation to B and T cell recovery early after transplant. During conditioning 16 patients received rATG (total 8 or 14 mg/kg) for *in vivo* TCD. This cohort was compared to 16 patients conditioned with hATG for *in vivo* TCD (total 20 or 40 mg/kg). Total serum ATG levels after alloSCT as measured with species-specific ELISA at 3 and 6 weeks after alloSCT were similar for the hATG (43 and 15 ug/mL) and the rATG cohort (41 and 21 ug/mL). Next, Specific *ex-vivo* reactivity with human B and T cells of the circulating ATG was measured by a flow-cytometry based method. In serum from hATG treated patients, a high specific anti-B cell reactivity was detected, which was dominant over anti-T cell reactivity, resulting in expansion of memory T cells which include EBV specific T cells prior to B cell recovery. In contrast, serum of rATG treated patients contained only marginal anti-B cell reactivity, but dominant anti-T cell reactivity resulting in early B cell expansion in the absence of T cell recovery. Interestingly, when tested directly out of the vial both ATG products showed similar dominant functional T cell reactivity over B cell reactivity as demonstrated using cell lineage specific flowcytometry and *in vitro* CDC assays.
In conclusion, after treatment with rATG, high levels of specific anti-T cell reactivity was found up to 9 weeks after allo-SCT, whereas anti-B cell reactivity was only detectable early after transplant, allowing B cell outgrowth and PTLD in the absence of EBV-specific T cell control. In contrast, *in vivo* TCD with hATG resulted in dominant persistence of anti-B cell reactivity up to 9 weeks after transplant. Preclinical *in vitro* testing of the reactivity patterns of both ATG products did not reveal these striking differences and is therefore not useful for predicting these *in vivo* effects.

**P513**

**Epstein-Barr virus driven lymphoproliferative disorders post allogeneic stem cell transplantation**

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Introduction: Epstein Barr Virus (EBV) driven post transplant lymphoproliferative disorder (PTLD) is an increasingly recognised complication of allogeneic stem cell transplantation. Changes in conditioning therapies and stem cell source (e.g. increased use of cord blood and unrelated donors) have influenced this increase. In 2009 we introduced routine PCR surveillance to detect EBV reactivation post transplantation. The aim of this study was to determine the incidence and risk factors for EBV reactivation, rate of progression to PTLD and impact of intervention with Rituximab.
Methods: The criteria for EBV surveillance were patients with steroid refractory GVHD and those who received ATG/Alemtuzumab as part of their conditioning therapy.
Results: Since 2009 we performed 187 allogeneic stem cell transplants (SCT), 106 were HLA matched sibling donors and 81 had unrelated donors (UD). Conditioning therapy was myeloablative (MA) in 58% and reduced intensity conditioning (RIC) in 42%.
Of the 108 patients who received MA conditioning, 13 (12%) developed steroid refractory GVHD and were treated with Thymoglobulin as second line therapy (10/13 UD). These patients also received one dose of pre-emptive Rituximab to prevent EBV driven PTLD. Despite this, EBV reactivation was detected in 6/13 patients (46%) treated with Thymoglobulin. Progression to PTLD occurred in 4/13 patients (31%). Rituximab therapy was given to all 6 patients and resulted in a clearance of EBV in 5/6 patients.1 patient (1% of all MA transplants but 8% of patients treated with Thymoglobulin) died from EBV driven PTLD.
79 patients underwent RIC based transplants. Fresenius ATG was included in the conditioning therapy in 47 patients (59%) and Alemtuzumab in 27 patients (34%).
EBV reactivation was detected in 8/79 (10%) of patients who had a RIC transplant and 7 patients required therapy with Rituximab. Of this cohort 5/79 (6%) developed PTLD - all were aged >50 years old and had unrelated donor SCT; 4 had been treated with ATG and 1 with Alemtuzumab. 2/8 patients died from EBV driven PTLD(25% of patients with detected EBV or 2.5% of all RIC transplants). Both patients were >60 years old with very early onset of PTLD(1 month from transplant) and a rapidly progressive course.
Conclusion: EBV reactivation is a common event following SCT that warrants prospective monitoring and early treatment intervention with Rituximab as the majority of the patients (11/13 in our cohort) who develop EBV driven PTLD can be salvaged.

**P514**

**Varicella-zoster virus infections after high-dose chemotherapy and autologous stem cell transplantation in children with malignancies**

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Varicella zoster virus (VZV) infection is a frequent complication of HDC followed by ASCT in children but few and conflicting data are published. The aim of this study was to determine frequency, risk factors and consequences of VZV infection after autologous HSCT in a large cohort of children and to adapt our therapeutic management.
We analysed prospectively collected data of children treated with HDC and HSCT in the pediatric department of Institut Gustave Roussy. Between January 1985 and July 2009, 1147 children received at least one course of HDC and ASCT. Most high dose regimen combined HD alkylating agents (Melphalan, Thiotepa, Busulfan). No VZV prophylactic treatment was administered post transplantation. 254/1147 patients (22.14%) developed 262 VZV events (22.84%). Their median age was 5.6 years (0-23).163 patients (64.17%) received a single course of HDC, 91 patients (35.83%) had a sequential HDC program and 5, 4, 3 and 2 courses were administered in 13, 3, 17, and 58 patients respectively. Chicken-pox and zoster represented 11.07 and 88.93 % of the VZV infections respectively. Eight double events including 2 varicella after zoster occurred. The median time of the VZV infection onset was 116 days post ASCT. 90.5% of cases occurred within the first year, but VZV infections occurred up to five years post-HSCT. Evolution was simple with acyclovir treatment in 88.17% of the patients. Complications consisted

mainly in post VZV neuralgia, 2 visceral dissemination resolved with treatment, 3 patients died of disease progression with a concomittent VZV infection. The drugs of the HDC regimen were a significant risk factor: Melphalan-Busulfan [RR = 2.3], BAM [RR = 2.22], Thiotepa (720 or 600 mg/m²) alone [RR = 2.13] or with Carboplatine [RR = 1.9] were associated with an increased risk of VZV infection. Hodgkin's disease [RR = 1.64]. was the only underlying disease correlated with an increased risk. Age did not influence the incidence of this complication.
In spite of the high incidence of VZV infections in these children receiving HDC and ASCT, the favourable outcome with a curative treatment with acyclovir, confirm the absence of benefits of a prophylactic strategy.

**P515**

**Successful high-dose chemotherapy with stem cell rescue for a child with resistant Burkitt's lymphoma and active chronic Varicella zoster virus infection**

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Background: Chronic varicella zoster virus (chronicVZV) infection is defined as atypical mucocutaneous wart-like and/or ulcerative lesions, persisting for at least 1 month. Infection with VZV peri hematopoietic cell transplantation (HCT) usually occurs post transplantation due to reactivation from earlier exposure to the virus.
We describe a 4.5 year old boy treated for resistant Burkitt's lymphoma who underwent successful autologous HCT although having active chronicVZV infection.
Patients and Methods: A 4.5 year old boy was diagnosed as suffering from Burkitt's lymphoma and treated according to the group-C LMB protocol. Due to resistance of the disease the treatment was switched to rituximab-ifosfamide-carboplatin-etoposide (R-ICE), on which he improved. During chemotherapy treatments, the patient developed primary VZV infection proven in immunofluorescence and cultures. IV acyclovir was started and switched to oral treatment after 7 days. Thus, vesicles turned to dry scars. A month later, still having dry scars, he developed new vesicles. Polymerase chain reaction (PCR) for VZV was also done in the blood and was found to be positive. Acyclovir treatment was returned again to IV. The patient also received immunoglobulins. Due to persistence positive blood VZV-PCR, treatment was changed to foscarnet. This turned the VZV-PCR negative. Nevertheless, while getting the chemotherapy of the conditioning he turned again positive in VZV-PCR and remained so along the whole period of transplant. On the skin all the lesions disappeared and no new lesions appeared.
Results: The patient underwent high-dose chemotherapy with carmustine, cytarabine, etoposide and melphalan with stem cell rescue while demonstrating positive VZV-PCR in blood (meaning VZV viremia) and on foscarnet. No major toxicity was documented. Time to recovery of the absolute neutrophil count above 0.5X109/l and 1.0X109/l was day +12 and +13, respectively. Time interval to platelets >25 $X10^9$/l was 33 days. He did not achieve platelets >$50X10^9$/l until his discharge. Imaging study with ultrasound demonstrated no evidence of disease and the patient was discharged home on day +43.
Conclusions: High-dose chemotherapy with stem cell rescue may be considered for pediatric patients with life-threatening resistant lymphoma although having viremia with VZV if they don't have any other manifestations of multi-organ involvement and they stay all the way on continues anti-viral treatment and recurrent IVIG.

**P516**

**The influence of adeno- and herpesviral infections on appearance of graft-versus-host disease on adult recipients of allogeneic stem cell transplantation**

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Graft-versus-host disease (GVHD) still remains a major obstacle in allogeneic haematopoietic stem cell transplant (HSCT) patients. Despite immunosuppressive treatment for GVHD, cell-mediated immunity is predictably low, placing the patient at risk for viral infections. Several studies have demonstrated that cytomegalovirus (CMV) and HHV-6 reactivations are correlated with the development of acute GVHD, but assessment of other herpesviruses remains still unclear.
The aim of our study was to evaluate the role of viral infections caused by adeno- and herpesviruses on GVHD during the early (<100 days post-HSCT) post-transplant period in group of adult recipients of allogeneic stem cell grafts. A group of 19 adult patients underwent allogeneic HSCT between 2007 and 2011 at the Department of Hematology, Oncology and Internal Medicine, Medical University of Warsaw and Department of Haemopoetic Stem Cell Transplantation, Institute of Hematology and Transfusion Medicine, Warsaw, Poland, which showed symptoms of acute GVHD, was chosen to this study. Nine of them received a matched unrelated and the rest a sibling graft. Quantitative real-time PCR assays were used to monitoring once a week adeno- and herpesviral (HSV-1/2, VZV, CMV, EBV, HHV-6, HHV-7, HHV-8) load prior two weeks and during acute GVHD signs.
Acute GVHD was observed as grade 0–I in 7/19 (37%) and grade II–IV in 12/19 (43%) patients. During considered period 7/19 (37%) patients have detectable CMV viremia. Of the 19, 14 persons (74%) had multiple viral infections, most often also including the detection of HHV-7 (10/14) or HHV-6 (5/14). Adenoviral DNA was detected in 21% (4/19) of the patients. Our data suggest that HHV-6 and HHV-7 reactivation occurs earlier than CMV viremia, and this viruses could implicate CMV reactivation and cytomegalovirus disease.
The impact of GVHD and its therapy on the immunity of the allograft recipient remains marked for a considerable period of time after transplantation. Among the adult HSCT recipients, adeno- and herpesviral reactivations/infections as an early clinical events post-transplant identify a group of patients with increased risk for treatment-related mortality. Our data shows evidence that HHV-7 DNAemia among with high CMV load increases the incidence of acute GVHD after HSCT.

**P517**

**Molecular decryption of an epidemic of group C adenovirus infections in paediatric recipients of haematopoietic stem cells**

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Introduction: Adenovirus infections are important causes of morbidity and mortality in units of hematopoietic stem cell transplants (HSCT). Several cases of infection with Adenovirus group C were observed between September and December 2010 in the pediatric unit of HSCT at the hospital Robert-Debré, 3 cases of adenovirus C1 and 5 cases of C2. Some of these infections have caused disseminated infections resulting in death. These infections may be due to reactivation or transmission between patients. The high sequence homology within the Adenovirus group C makes it difficult to exclude the circulation of different strains.

Objectives: Study the variability of different strains of group C adenovirus isolated from patients and the environment in order to demonstrate transmission between patients.
Materials and Methods: The variability study was performed on strains from samples of patients of the unit positive with adenovirus C1 (C1A, C1B and C1C) and C2 (C2A, C2B, C2C, C2D and C2E) and samples from the environment positive with adenovirus C2 (C2env). Samples from patients infected with adenovirus C1 and C2 from other sites and sequences from GenBank® were also included as controls. Phylogenetic analysis has focused on the genes encoding the variable regions of the viral capsid HVR7 and fiber, and the non structural regions E1 and E3.
Results: Analysis sequences of fiber, E1 and E3 showed no significant differences between the sequences of patients. Analysis sequences of HVR7 and concatenated sequences have identified a circulation of different strains of C1 and C2. The analysis shows two independent events among the three cases of C1 and among the five cases of C2 it shows the independence of the strain C2B while the others were strongly associated compatible with a nosocomial transmission.
Conclusions: The differentiation of strains of adenovirus group C requires analysis of several regions of the genome. Our results show that several cases may be associated with independent events. Our results also show a strong association of epidemic strains compatible with a nosocomial transmission. However, the high sequence homology of C2 variants emphasizes the need to confront the epidemic data to phylogenetic data in order to establish formal links between cases. These data show the importance of monitoring patients at risk and the need to reinforce hygienic measures to control the spread of virus in the environment when positive patients are detected.

**P518**

**Adenovirus infection in a paediatric HSCT recipient – single-centre experience**

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Adenovirus (AdV) infections represent serious complication in the immunocompromised host. Moreover when adenovirus infection occurs,the infection can easily spread to other patients – either directly (e.g. by the infected medical staff) or indirectly by presence of adenovirus infectious particles in the environment and by transfer of these particles to another host.
Between I/2003 and XI/2011, we have tested 3445 samples from 231 patients (pts.) for presence of adenoviruses of our total 259 allogeneic HSCT recipients transplanted during this period. There were 2671 whole blood samples from 219 pts., 378 stool samples from 66 pts. and 396 another biological samples from 59 pts. In the time of outbreaks, we have also tested swab samples from the environment. Detection was performed by RQ-PCR according to the described assay detecting mainly the AdV serotype group A-C. AdV infection was detected in 155 whole blood and 157 stool samples from 45 pts. The infection was detected by the median of 46 days after HSCT and continued for median of 19 days (range 0-291). AdV was detected both in whole blood and stool in 17 pts., while in blood and stool only, there were 18 pts. and 8 pts. respectively. AdV was detected only once in 15 pts. Time of AdV detection in the stool did not precede detection in the whole blood. Log of the detected quantity in 1 ml of whole blood and stool was 3.82 (range 1.93-7.99) and 5.90 (range 2.07-10.61) respectively. Most frequently observed clinical signs of the AdV infection were nausea and diarrhea. In 18 children cidofovir treatment was started with good response in all of them and toxicity in 1. Five children deceased while AdV infected, in 2 we emphasize AdV to be causative agent.
AdV infection was usually observed in the outbreaks lasting for many weeks, starting in III/2003 (3 pts.), V/2006 (8 pts.), XII/2008 (8 pts.), V/2010 (6 pts.) and X/2010 (8 pts.). In most of the children AdV 31 was detected, while in the rest AdV 2 and 5 were detected. During these outbreaks, AdV DNA was detected on the surfaces in the transplant ward environment too (e.g. on the door handles, phones, keyboards etc.).
AdV infection was detected in 22.8% of our HSCT patients. We did not found yet any clear predictor of AdV infection among our patients. Our data suggests the necessity of quick and strict barrier approach to stop the AdV spreading, as well as necessity of more studies concerning the impact of different AdV serotypes on the outcome of the patient.
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**P519**

**Adenovirus-specific T-cell transfer after haematopoietic stem cell transplantation is feasible and can induce immunological response in adult recipients**

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Background: Infectious diseases are still one of the major problems after allogeneic hematopoietic stem cell transplantation (HSCT). Infrequent but emerging viral infections, such as Adenovirus (ADV) infections are often associated with a high mortality in immunocompromised patients (pts) and are difficult to treat with antiviral agents currently available.
Objectives: In this analysis, we show the results of three adult pts treated at our transplantation unit with ADV-specific donor t-cell transfer (ADV T-cells) suffering from ADV viremia with disease symptoms or disseminated ADV infections.
Patients and Methods: Pts treated at our transplantation unit between Jan 2007 and Nov 2011 were enrolled into this analysis. All pts were serologically screened for ADV antigens at the beginning of the treatment. In case of clinical symptoms, quantitative ADV PCR was performed in the symptom specific specimens and in blood samples. In uncontrolled ADV infections, or viremia, we transferred ADV T-cells in addition to the common antiviral agents. ADV-specific T-cells were monitored in two patients by interferon gamma elispot assay.
Results: 515 pts were treated between Jan 2007 and Nov 2011. In 25 pts (4,8%) we detected ADV by serology or PCR in different specimens. Three pts showed clinical symptoms related to ADV, which could not be resolved with the common available antiviral agents. All three pts suffered from diarrhoea when ADV were first detected in stool samples at day +91, day +3, and day +39 after HSCT, respectively. ADV T-cells were transferred at day +141, day +43, and day +61 after HSCT, respectively. No ADV was detected by PCR after ADV-T-cells in both pts with viremia. The application of ADV T-cells was tolerated well in all pts. There was no clinical association with the application of ADV T-cells and Graft-versus-Host Disease (GvHD). Two pts died due to severe GvHD and/or other infectious complications at day +179 and day +73 respectively (Table I). In one patient a long-lasting immunological response more than two years after adoptive transfer could be observed.
Conclusions: Adoptive ADV specific T-cell transfer is feasible in adult HSCT recipients suffering from severe ADV infections. We could demonstrate the clinical efficiency with the clearance of ADV viremia by ADV T-cells and a long-lasting immunological response without any side effects in one patient.

[P519]

Table I. Patients and treatment characteristics

| *Patient no.* | *1* | *2* | *3* |
|---|---|---|---|
| Age (years) | 20 | 49 | 18 |
| Gender | male | male | female |
| Disease | SAA | T-NHL | SAA |
| Donor | MUD | MRD | MUD |
| Graft | BM $1,6 \times 10^8$ MNC per kg/BW | PBSC $7,4 \times 10^6$ $CD34^+$ cells per kg/BW | BM $2,2 \times 10^8$ NC per kg/BW |
| Conditioning | TNI 2x4Gy – Flu30/4 – ATG30/4 – Cy50/4 | FLAMSA – TBI 4Gy – Cy40/2 – ATG 10/3 | TNI 2x4Gy – Flu30/4 – ATG30/4 – Cy50/4 |
| Immunosuppression | CSA/Siro | Siro/MMF | CSA/Siro |
| Day of ADV detection/ body fluid | +91/stool | +3/stool | +39/stool |
| ADV viremia | no | yes | yes |
| ADV in other body fluids | Urine | Throat swab, BAL | Throat swab, BM |
| Antiviral agents prior to ADV T-cells | Acyclovir, Cidofovir, Ribavirin | Acyclovir, Ganciclovir, Cidofovir, Foscarnet, Ribavirin | Acyclovir, Cidofovir |
| Intravenous Immunglobulin | yes | yes | yes |
| GvHD prior to ADV T-cells | Gut, liver stage IV | Skin, gut stage IV | no |
| Day of ADV T-cells | +141 | +43 | +61 |
| Symptoms at time of T-cell transfer | Diarrhoea, fever, hematuria | Pneumonia with artifical rspiration, sepsis | no |
| ADV viremia clearence | – | yes | yes |
| Follow-up (days) | 179 | 73 | 726 |
| Outcome | died (GvHD) | died (sepsis) | alive |

Abbreviations: SAA – severe aplastic anemia; T-NHL – T-cell non-Hodgkin Lymphoma; MUD – matched unrelated donor; MRD – matched related donor; BM – bone marrow; MNC – mononuclear cells; PBSC – peripheral blood stem cells; NC – nuclear cells; BW – body weight; TNI – total nodal irradiation; Gy – gray; FLU – fludarabine; ATG – anti-thymocyte globulin; Cy – cyclophosphamide; FLAMSA – fludarabine, amsacrine, cytarabine; TBI – total body irradiation; CSA – cyclosporine; Siro – sirolimus; MMF – mycophenolatemofetil; ADV – human adenovirus; BAL – brochoalveolar lavage; ADV T-cells – ADV-specific donor t-cell transfer; GvHD – graft-versus-host disease;

**P520**

**Clearance of adenovirus load in a recipient of mismatched stem cell transplantation after adoptive transfer of adenovirus-specific T-cells from a haploidentical donor**

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Viral reactivations are serious complications in recipients of T-depleted stem cell transplantation (SCT). Adenovirus (ADV) is known to be the most dangerous viral pathogen in this context, being responsible for a high mortality rate in the pediatric setting. Antiviral agents are nonspecific and therefore show limited efficacy together with significant toxicity. Immune reconstitution is to be considered the key factor in viral control after SCT. Though not fully unrevealed, immune response to viral activation seems to rely predominantly on T-cell expansion. Therefore cellular immunotherapy was successfully developed to allow adoptive transfer of virus-specific cytotoxic T-lymphocytes (CTL).

We present the case of a 16 year-old boy developing ADV reactivation in blood as measured by Polymerase Chain Reaction during the preparatory regimen for haploidentical T-cell depleted SCT from father.

Three days before SCT, ADV was detected in blood (41,000 copies/mL), antiviral chemotherapy was started with weekly doses of cidofovir 5mg/kg. Analysis of the patient's blood in the weeks after SCT showed severe reduction of T-cells and specifically, absence of IFN-gamma secreting T-cells after *in vitro* stimulation with ADV peptides. ADV load peaked at 400,000 copies/mL without, however, signs of clinically overt disease.

To transfer immunity against the virus, ADV specific CTLs were selected from the haploidentical donor through clinical-grade IFN-gamma secretion assay as published by Feuchtinger. On day +20 after SCT $2.3x10^3$/kg IFN-gamma specific T-cells were infused without side effects and with no subsequent signs of GvHD.

At that time point the patient exhibited <10 T-cells/microL and a viral load of 60,000 copies/mL under regular antiviral therapy. After CTL administration we observed a prompt and lasting increase of T-lymphocytes. ADV load decreased and became negative 19 days after CTL infusion. By that time a positive population of ADV reactive T-cells was detected in the patient's blood, with a frequency of 17%, chimerism being full donor.

The present case report shows that haploidentical donor-derived ADV specific CTLs can boost T-cell reconstitution in recipients of T-depleted SCT. Of importance ADV specific T-cells could be detected in the recipient only after adoptive transfer and a high *in vivo* expansion rate of these cells was documented. We believe these cells together with conventional anti-viral therapy played a major role in ADV infection control.

**P521**

**Early diagnosis and anti-viral intervention at the onset of haemorrhagic cystitis has considerable impact on patient outcome after allogeneic haematopoietic stem cell transplantation in children**

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Background: Hemorrhagic cystitis (HC) is a serious complication after hematopoietic stem cell transplantation (HSCT). Salient recent evidence has been revealed the pathogenic role of JC/BK and adenovirus infection or reactivation. Early diagnosis and adequate treatment are definitely of remarkable importance on preventing the development of subsequent life-threatening complications.

Objectives: To describe the clinical course of HC after HSCT and to set out the most effective treatment combination and optimal timing in prospective substantive cases.

Methods: Eight patients developing HC of 34 patients undergoing HSCT between 2010-2011 at our center have been analyzed retrospectively. The familial and previous individual anamneses, underlying primary diseases, clinical, laboratory and microbiological data were recorded and reviewed for.

Results: Median age of the children was 9.6 ± 5.3 years. Primary diseases were acute lymphoid and myeloid leukaemia, chronic myeloid leukaemia, aplastic anaemia, Glantzmann thrombasthenia and thalassaemia. The onset of HC was observed at the 24 ± 7 posttransplantational day and bleeding was detected for 34 ± 30 days along. Preceding, all patients received cyclophosphamide treatment in the conditioning period. One of the patients developed grade 3 and two of them grade 4 HC. Cidofovir treatment was initiated in 7 cases after detecting urinary bleeding. Five of the patients showed complete recovery. In one case the hemorrhagic cystitis was accompanied with fatal rapidly progressive interstitial pneumonitis in the engraftment period, while in two cases subsequent CMV infection were detected leading to graft rejection and finally uncontrollable fatal interstitial pneumonitis after temporary resolution of the HC.

Conclusion: For selection of patients susceptible to HC, routine pretransplantational screening for urine and blood stream JC/BK and adenovirus infection are suggested. Our observational results imply that early anti-viral treatment in the 15-25 days posttransplantational period - even in case of high fever, still without clinical or laboratory signs of HC - can be of crucial importance on preventing graft rejection and consecutive life-threatening complications, which considerably prejudice patient outcome after HSCT. Indeed, pretransplantational microbiological positivity may give rise to preventive administration of cidofovir to improve the response achievable by traditional treatment strategies.

**P522**

**BK cystitis in HSCT patients – the search for effective prophylaxis**

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Background: BK virus (BKV) reactivation in patients undergoing allogeneic stem cell transplant is known to cause late-onset haemorrhagic cystitis (HC). BKV replication has been shown to be inhibited by flouroquinolones *in vitro.* We performed a prospective study to test the effectiveness of a prophylaxis protocol incorporating ciprofloxacin in reducing morbidity from BKV reactivation.

Objectives: Our primary objective was to determine whether this protocol could decrease rates of significant haemorrhagic cystitis (grade ≥2) and/or renal impairment in our allogeneic transplant patients. Our secondary objective was to analyze the rate of development of BK viraemia and viruria and its associated complications in our transplant population.

Methods: From September 2009 onwards, we monitored BK viral load in the urine and blood together with urine FEME from day 0 then weekly from day 14 until engraftment. We used ciprofloxacin 500mg twice daily as BKV prophylaxis from day of admission for transplant to day 56 with escalation to levofloxacin if viruria or viraemia increased. HC of ≥ grade 2 was treated with various combinations of leflunomide, intravesicular and/or intravenous cidofovir and immunosuppression reduction.

We prospectively analyzed rates of HC and renal failure among our patients after implementation of this protocol, from September 2009 to August 2011. This was then compared with a historical control of allogeneic transplant patients from October 2007 to August 2009.

Results: The prospective group had 73 patients and the historical control 59 patients, both with similar median ages of 45 years. The groups were comparable in terms of haematological profile, donor source and conditioning regimens. Giving ciprofloxacin prophylaxis and escalating treatment as per protocol made no difference to rates of grade ≥ 2 HC or renal impairment when compared to the historical control.
BK viruria and viraemia were detected in 75% and 38% of patients in the prospective group. There was a significant and independent association between the presence of BK viraemia, BK urine load of 5.5 log and grade ≥ 2 HC. Mortality rates in the 2 study populations were similar.
Conclusions: In our study, strict administration of ciprofloxacin prophylaxis for BKV was not effective in reducing the rates of clinically significant HC; this is at variance with a recent smaller study that demonstrated benefit. The search for effective porphylaixs for BK haemorrhagic cystitis should continue.

**P523**

**Prospective monitoring of urinary BK and JC shedding by quantitative PCR in recipients of allogeneic haematopoetic stem cell transplants is useful to anticipate the development of haemorrhagic cystitis**

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Background: Polyomavirus infection is highly prevalent in humans. In HSCT, BK virus (BKV) has been associated with the development of HC, whereas the role of the JC virus (JCV) is unclear.
Materials and Methods: We studied 436 urine samples from 48 pts who underwent 50 allo-HSCT between January 2010 and November 2011. Thirty-one were male (64.5%) and 17 female (35.5%). Median age was 48 years old (range: 6-65). Baseline disease was: acute leukemia (n=13), myelodysplastic syndrome (n=4), myeloproliferative disorder (n=2), multiple myeloma (n=20), lymphoproliferative disorder (n=8) and Fanconi anemia (n=1). Twenty-six underwent matched siblings HSCT (52%), and the remainder matched unrelated-donor HSCT (48%). Four pts received reduced intensity conditioning (RIC) regimens (8%). SC sources were peripheral blood (n=27), bone marrow (n=19), and umbilical cord blood (n=4). BKV and JCV qPCR were performed in urine samples collected at admission for transplant and weekly until discharge.
Results: Eight pts (16%) developed HC. BK viruria was demonstrated in 36 pts (72%) at day +7 as a median (range: -7 to +110); only one patient receiving a RIC regimen had urinary BKV excretion. Median BKV load was 6.8 log10 copies/mL in the group of pts who developed HC and 3.2 log10 copies/mL in those who did not (p=0.036). Pts who had clinical HC showed higher viral load at first detection (log10 4.17 *vs* 2.7 copies/mL; p=0.003) and at maximum viral load (log10 8.18 *vs* 3.2 copies/mL; p=0.013) than pts without HC. There was a significant correlation between the first and the maximum BKV load (R=0.445; p=0.009). The median duration of BKV excretion was 230 days in pts with HC and 77 days in pts without HC. JC viruria was seen in 26 pts (52%), being more frequent when peripheral blood was used as the SC source (p=0.01). Median time for first JCV detection was day -1.5 (range: -11 to +347). Median duration of JCV excretion was 34 days in pts who developed HC and 24 days in pts who did not. A significant correlation was found between BKV and JCV maximum loads (R=0.451; p=0.04).
Conclusions: 1) JC and BK shedding in urine were frequent in our series of allo-HSCT recipients. 2) High JCV and, particularly BKV load correlated with the development of HC. 3) We suggest that high load of BK excretion should be considered a warning sign for the development of HC in allo-HSCT pts, and preemptive therapeutic interventions need to be further investigated.

**P524**

**Graft failure after lamivudine resistant variant HBV infection: combined antiviral therapy with entecavir and tenofovir improve engraftment in allogeneic HCT**

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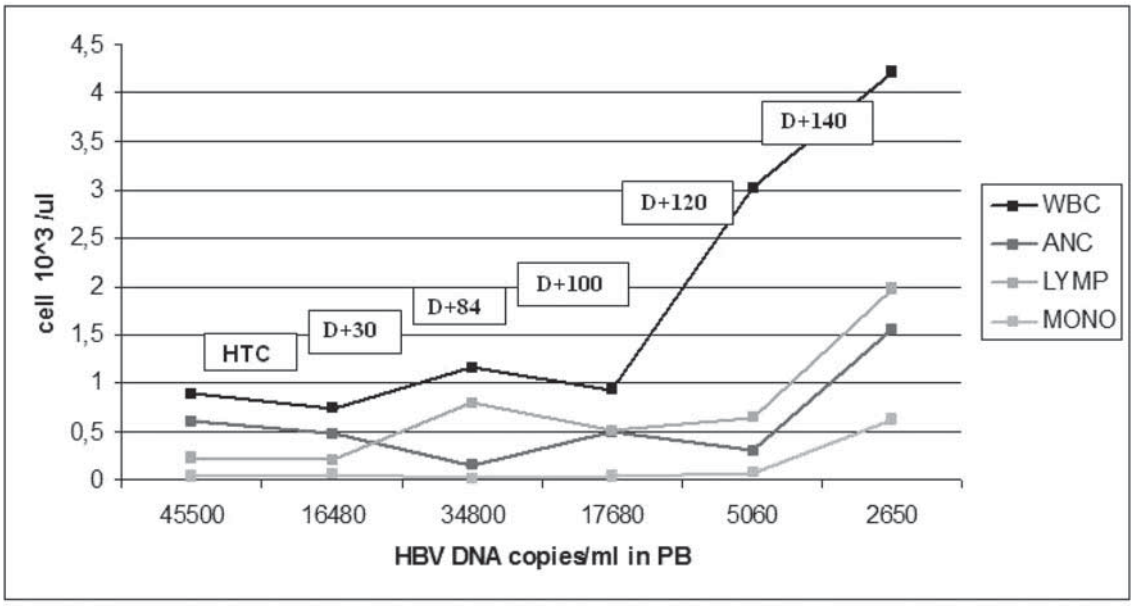
After allogeneic Haematopoietic Cell Transplantation (HCT) several virus may reactivate exerting negative effect on post-engraftment haematopoiesis. Although Hepatitis B virus (HBV) can infect bone marrow (BM), usually in patients affected by HBV anti-viral therapy with Lamivudine protects hematopoietic cells.
HBV Lamivudine resistant (LR-HBV) variants have been recently described, their effect on post-HCT engraftments is still not defined.
We describe a case of BM infection with LR-HBV (YMDD) variant associated with prolonged marrow aplasia after HCT.
A 45 years old man with diagnosis of T cell Lymphoblastic Lymphoma, after three lines of therapy including autologus HCT, received HLA matched HCT from unrelated donor. He had a history of HBV infection, therefore he was treated with Lamivudine therapy since the diagnosis of Lymphoma. Two months before HCT was detected an increase of HBV title with more than $1x10^6$ copies in peripheral blood (PB). Patient was then treated with Entecavir plus Lamivudine; when a LR-HBV variant was detected in PB, treatment was continued with Entecavir alone. HBV DNA copies were 45.500 UI/ml at the time of HCT.
Conditioning regimen included Thiotepa, Fludarabine, Cyclophosphamide and Treosulfan; he received 3.1 $x10^6$/kg CD 34 cells. Mycophenolate Mofetil and Tacrolimus were used as graft versus host disease (GVHD) prophylaxis.
The patient engrafted with day +30 full donor chimerism. HBV copies were 16,480 UI/ml.
At two months after transplant concomitantly with a CMV reactivation he experienced a tri-linear cytopenia that persisted despite Foscarnet therapy had cleared CMV in 3 weeks.
On day + 84 a marrow biopsy was performed and while no copies of CMV were detected a massive HBV infection was demonstrated in BM and at same time the copies in PB increased to 34,800 UI/ml.
To this light an association of Entecavir plus Tenofovir was started.
Few weeks after the start of this combination therapy a progressive decrease of HBV DNA copies was observed (3050 UI/ml) with a concomitant increase of white blood cell count up to normal values (Figure 1). Grade 3 thrombocytopenia and anaemia persisted longer.
We describe a rare case of BM infection with the YMDD (LR-HBV) variant suggesting not only that this infection might be associated with poor engraftment or graft failure but also that the association of double anti-viral therapy (Tenofovir and Entecavir) deserves further investigation in this setting.

[P524]




Relation between progressive decrease of HBV DNA copies in PB and rising of leukocytes

**P525**

**Immunochromatography method was useful in prompt diagnosis of potentially fatal norovirus gastroenteritis after haematopoietic stem cell transplantation**

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Background: Norovirus-gastroenteritis (NV-GE) is considered as a highly transmittable disease that can lead to fatal outcomes in vulnerable populations. Therefore, prompt detection of norovirus in stool specimens is important for patients who undergo hematopoietic stem cell transplantation (HSCT). The commercially available immunochromatography kit (Denka Seiken, Tokyo, Japan) is a diagnostic tool that can easily and rapidly detect norovirus antigens with high specificity and relatively less sensitivity compared with reverse transcription polymerase chain reaction (RT-PCR). Here, we report an analysis of patients with NV-GE in our HSCT unit using the immunochromatography method.
Patients and Methods: We prospectively examined stool specimens to detect norovirus antigens in patients who developed diarrhea in our HSCT unit between December 2008 and June 2011. We also retrospectively examined stool specimens which had been collected for Clostridium difficile (CD) toxin test and frozen at –40°C between January 2007 and November 2008.
Results: Norovirus was detected by the immunochromatography method in 11 patients among the HSCT recipients, and in 1 patient who died before HSCT because of disease progression. The median age of the 12 patients was 56 years (range, 29-66). The median duration of symptoms was 30 days (2-134). Among the 11 patients who developed NV-GE after HSCT, primary disease included lymphoma (6 patients), acute leukemia (4 patients), and multiple myeloma (1 patient). One patient developed NV-GE after autologous HSCT, and 10 patients after allogeneic HSCT. Among the 10 allo-HSCT recipients, 5 patients received myeloablative conditioning and 5 received reduced-intensity conditioning before allo-HSCT. The median time from HSCT to the onset of symptoms was 36 days (4-93). The median time from HSCT to diagnosis of NV-GE was 37 days (11-101). At diagnosis of NV-GE, all allo-HSCT recipients were given immunosuppressive agents. No patients died of NV-GE, and 6 died of other causes (disease progression, 4; Graft-versus-host disease, 1; multiple organ failure, 1). There was no outbreak of NV-GE.
Conclusions: Our results suggested that this method is helpful in the differential diagnosis of patients with diarrhea after HSCT and enable us to take an appropriate and prompt preventive measure. In the future study, validation with RT-PCR and immunochromatography method is warranted among the immune-compromised populations.

**P526**

**Infection of lower respiratory tract with human rhinovirus type C in a paediatric cord blood transplant recipient**

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Introduction: Human rhinoviruses (HRV) are a commonly identified cause of upper and lower respiratory tract infections (U/LRTI) in hematopoietic stem cell transplant (HSCT) patients. Recently, a newly discovered HRV, HRV type C (HRV-C), has been identified in patients with severe LRTI. There is currently a lack of information on the incidence or pathogenicity of HRV-C in the pediatric HSCT population. We present a case of HRV-C LRTI in a pediatric patient with monosomy 7 juvenile myelomonocytic leukemia (JMML).
Clinical Summary: A 6-month-old boy was diagnosed with monosomy 7, JMML in July 2010. Due to significant tumor burden he required chemotherapy, decompressive surgery with splenectomy, and ventilator support pre-transplant. In December 2010 he received a reduced intensity transplant with Melphalan and Fludarabine and 5/6 DRB1 antigen mismatch cord blood. Within 1 month of transplant, nasopharyngeal swab was positive by PCR for HRV. He remained positive for HRV with

and without symptoms over the next 5 months, and during that period a broncheoalveolar lavage (BAL) was positive for HRV, indicating LRT involvement. BAL also showed presence of Klebsiella and Enterobacter Asbuirae. In June 2011 he developed both Human Herpes Virus 6 (HHV6) viremia with pneumonia, and BAL was positive for both HRV and HHV6. HHV6 viremia resolved with cidofovir and foscarnet therapy, but respiratory HHV6 infection persisted. Determination of HRV type by conventional PCR with HRV-specific primers targeted at the 5' noncoding region identified HRV-C serotype 025. In this setting of respiratory co-infection with HRV-C and HHV6 the patient relapsed and, in August 2011, underwent a second transplant with 5/6 DRB1 antigen mismatch cord blood and myeloablative Busulfan, Cyclophosphamide and Melphalan conditioning with maintenance foscarnet therapy as it was the only cure of his disease. Unfortunately disseminated HHV6 infection developed 1 month later and he succumbed to renal and respiratory failure and encephalitis 2 months post second transplant.
Conclusion: This finding of LRT HRV-C infection in a pediatric HSCT patient with bacterial and HHV-6 co-infection demonstrates the need for increased awareness and understanding of potential respiratory complications of HRV-C infection, including the possibility of increased susceptibility to and persistence of co-infecting pathogens.

## Acute Graft-versus-host Disease

**P527**
**A multicentre retrospective analysis on pentostatin as salvage therapy of severe steroid refractory acute graft-versus-host disease of the gastro-intestinal tract**
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Steroid refractory acute GvHD of the GI tract is a life threatening complication after allogeneic SCT. Pentostatin has shown efficacy as salvage therapy in this situation in single center studies. Here we report on the experience with pentostatin in severe steroid-refractory aGvHD of the GI tract at 8 centers.
Patients: 128 patients were treated with pentostatin due to grade III (61) or IV (67) intestinal steroid-refractory aGvHD between 2000 and 2011. Pentostatin was infused at a dose of 1 mg/qm x 3 days (1-4 cycles). Steroids and CNI were continued. Response was classified as complete (CR) or very good partial (VGPR). The underlying diseases were AML (72), ALL (16), MPN (6), lymphoma (12), MDS (12), multiple myeloma (9) and aplastic anaemia (1). Patients were transplanted from matched related (39), matched unrelated (57) or mismatched donors (32). Patients received pentostatin as 1st line salvage (112) or beyond 1st line salvage therapy (16).
Results: 55 patients (43%) responded after pentostatin. 42 patients (33%) achieved CR, 13 patients (10%) VGPR. Median survival was 104 days; 2-year and long term survival rates were 28 and 21% (median follow up: 45 months). Patients who had been transplanted from a matched related donor had a significantly (p=0.04) higher probability of survival in comparison with patients with other donors (2-year survival: 38 *vs.* 21%, long term survival 35 *vs.* 8%). 53% (20) of these patients responded. Out of the 112 patients who were treated with pentostatin as first line salvage therapy 15 received simultaneously additional immunsuppressive salvage therapies (e.g. infliximab, MSC or ECP). None of these patients survived. 47 patients without CR after one cycle of pentostatin received further immunosuppressive salvage treatment: 29 were treated with 1-3 further cycles of pentostatin. 18 received pentostatin plus simultaneous or subsequent additional immunsuppressive therapies. In both groups survival rates were identical.
Conclusions: The outcome after salvage therapy of III/IV° steroid-refractory intestinal aGvHD with pentostatin is within the range as reported for other salvage approaches. In this critical clinical situation pentostatin has superior characteristics: a sustainable effect, moderate toxicity and cost-effectiveness. Moreover, this analysis suggests that the outcome cannot be improved by the application of more than one immunosuppressive salvage drug in addition to steroids and CNI or by second line salvage approaches.

**P528**
**Toll-like receptor pathway gene polymorphisms are associated with acute GvHD risk after related and unrelated haematopoietic cell transplantation**
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Objectives: Graft-versus-host disease (GVHD) remains one of the most challenging obstacles to successful allogeneic hematopoietic stem cell transplantation (allo-HSCT), and limits the use of this important procedure. Therefore, defining variables that predispose to this event is of critical importance. Toll-like receptors (TLRs) are transmembrane proteins on the surface of immune cells that detect "microbe-associated molecular patterns" from a variety of organisms. There is increasing evidence for a role for TLRs in the pathogenesis of acute GVHD (aGVHD). We hypothesized that gene polymorphisms in TLR pathway in donors or recipients may affect the risk of aGVHD.
Methods: Ten single nucleotide polymorphisms (SNPs) in the TLR1, TLR2, TLR3, TLR8 and TLR9 genes were analyzed in 2 independent cohorts. The initial cohort consisted of 138 pairs of patients and their unrelated donors (URDs). The second cohort consisted of 102 pairs of patients and their HLA-identical sibling donors.
Results: We found that two SNPs in donor side, TLR9-1174 G/A (rs352139) and TLR9 +1635 C/T (rs352140), influenced the risk of aGVHD. The association was particularly strong in the URD transplantation cohort. Multivariate analysis confirmed that an unrelated donor with the TLR9-1174 mutant genotype (AA/AG) was an independent risk factor for development of aGVHD (P=0.055, RR=2.115). In contrast an unrelated donor with the TLR9 +1635 mutant genotype (TT) was protective (P=0.006, RR=3.556). Myeloablative conditioning (P=0.005, RR=3.601) and donor female and recipient male (P=0.04, RR=1.577) also significantly contributed to the development of aGVHD. The same effect was observed in the sibling transplantation cohort, although the incidence of clinically significant aGVHD in this cohort was low overall and the association was not statistically significant. The incidence of aGVHD was not affected by polymorphisms of the TLR1, TLR2, TLR3 and TLR8 genes in either recipients or donors.
Conclusion: Current evidence suggests that in addition to HLA matching, polymorphisms in donor and recipient non-HLA genes affect transplantation success by regulating alloimmune responses. These results provide the first report of an association between donor TLR9 gene polymorphic features with the risk of aGVHD, which are located within the promoter region and coding polymorphisms and may influence transcriptional regulation and the amino acid exchange of the TLR-9 gene.

**P529**
**The effects of immunosuppressive drugs – mycophenolate mofetil and cyclosporine A – on umbilical cord blood and peripheral blood T-cells**
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Haematopoietic stem cell transplantation (HSCT) is a common treatment of haematological diseases. Cord blood (CB) is increasingly used as source of stem cells (SC), as it allows

transplantation with a lower HLA match. Prophylaxis drugs such as mycophenolate mofetil (MMF) and cyclosporine A (CsA), which are often used together after HSCT to prevent graft versus host disease (GvHD), have been studied for their immunosuppressive effects on peripheral blood mononuclear cells (PBMCs), but so far little is known about their effects on CB mononuclear cells (CBMCs). Moreover, it has been shown that the administration of MMF after CB transplantation delays neutrophils engraftment. Therefore, it is crucial to understand better the effects of MMF and CsA on CB cells.
Using a flow cytometry approach, the effects of MMF, CsA and the combination of both drugs, on cell death, T cell subsets and cell activation were studied on resting and activated CBMCs and PBMCs. PBMCs and CBMCs reacted differently to the addition of these drugs. MMF delayed, but did not abrogate T cell proliferation and activation. Moreover, the use of MMF did not increase the cell death of activated CB cells and preserved the regulatory T cell (Treg) population, which could be important to prevent GvHD. None of these effects were seen when using CsA. Furthermore, CsA seemed to inhibit CD4 and CD8 re-expression after activation. When using the two drugs together, the effects on proliferation, activation and CD4/CD8 populations persisted except for the preservation of the Treg population.
CBMCs produced lower levels of various Th1 and Th2 cytokines than PBMCs. However, when activated, CBMCs secreted superior levels of IL-6 than activated PBMCs. The level of secretion of every cytokine tested was decreased with the addition of CsA while the level of IL-2 production was not changed by the addition of MMF.
In addition, MMF reduced colony forming units (CFUs) formation from CBMCs in a non-permanent manner, which correlates with the delay, not the abrogation, of SC engraftment using MMF as prophylaxis treatment.
In conclusion, we showed that MMF and CsA have differential effects on CBMCs and PBMCs, and that MMF had broad effects on T cells, some of them being possibly beneficial in the HSCT setting using CB as a source of SC. Some of these advantages were lost with the addition of CsA. But, the fact that MMF seems to delay SC engraftment raises the question of its potential effect on SC.

**P530**

**Many days at home during the neutropenic phase after allogeneic haematopoietic stem cell transplantation decreased acute graft-versus-host disease**

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After allogeneic hematopoietic stem-cell transplantation (HSCT), patients living within one and a half hours' driving distance to the unit were given the option of treatment at home after HSCT. An experienced nurse visited the patient every morning and a phone-call from a physician was given in the afternoon.
One-hundred and forty-six patients treated at home during the neutropenic phase were compared to matched hospital controls, coming from outside Stockholm. Because good oral nutrition was correlated to reduced acute GVHD, oral intake was intensified in patients treated in the hospital from September 2006 (new controls). We compared four groups; old home-care (before September 2006, n=76), new home-care (from September 2006, n=70), old (n=76) and new controls (n=70).
The cumulative incidence of acute GVHD grades II – IV was 15% in the old home-care group, which was significantly lower as opposed to 32–44% in the new home-care group ($p<0.001$) and the two control groups ($p<0.03$). Cumulative incidence of chronic GVHD was between 35 and 41% in the four groups.
Transplant-related mortality was between 10 and 24% in the four groups (n.s.). No patient died at home. Relapse probability was similar in the four groups. Oral nutrition (kcal/kg/day) during the first three weeks was significantly worse in the old controls, as opposed to all other groups ($p<0.01$). Among the old controls, oral nutrition was median 18.5 kcal/kg/day, as opposed to 23.6 kcal/kg/day in the new controls ($p=0.002$). Oral nutrition ($p=0.02$) and days at home ($p=0.005$) were correlated to low grade of acute GVHD. The new home-care patients spent fewer days at home ($p=0.002$). In multivariate analysis, GVHD grades 0-I was associated with home care (HR 2.46, $p=0.02$), days spent at home (HR 0.92, $p=0.005$), but not to oral nutrition (HR 0.98, $p=0.13$). Despite improved oral intake in patients treated in the hospital more recently, acute GVHD did not decrease. The old home-care patients had a decreased risk of acute GVHD, which probably is due to a long time spent at home.
Five-year survival was 61% in the home-care group as compared to 49% in the controls ($p=0.07$).
Conclusion: Home care is safe. Home care and many days spent at home were correlated to a low risk of acute GVHD.

**P531**

**Deletion of methotrexate from a triple immunosuppression prophylaxis reduces haematological toxicity and inpatient stay with no impact on graft-versus-host disease in patients with haematological malignancies treated with an allogeneic transplantation**

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Objectives: To analyse haematological recovery, duration of inpatient stay and incidence of acute and chronic graft versus host disease (GVHD) when deleting short course methotrexate (MTX) from a triple immunosuppression prophylaxis program including alemtuzumab in patients with haematological malignancies treated with a reduced intensity allogeneic stem cell transplantation (RIC-allo).
Patients and Methods: Thirty-seven consecutive patients allografted (either from a HLA identical sibling donor or from a matched unrelated donor) in a single institution over a 18 months period (January 2010–May 2011) were retrospectively analysed. The main indications for RIC-allo were acute myeloid leukaemias (21; 56.7%), lymphoma (7; 18.9%), myeloproliferative neoplasms (4; 10.8%) and myelodysplastic syndromes (4; 10.8%). Twenty-five patients received the combination of Campath 1H + cyclosporine A (CsA) / tacrolimus (TAC) + short course MTX as GVHD prophyalxis after RIC-allo while twelve, the combination of Campath 1H + CsA / TAC at the same doses. Both groups were comparable in terms of sex, age at the time of RIC-allo, underlying disease and disease status at transplantation, type of donor and source of stem cells, risk factors and time interval between diagnosis and RIC-allo.
Results: Campath–CsA/TAC group had a significantly quicker haematological recovery after RIC-allo both in terms of neutrophils and platelets; 19 days *vs* 22.5 days and 13.6 days *vs* 25.5 days, respectively. In addition, the length of hospital admission was significantly lower in the group that did not receive MTX (23.25 days *vs* 35.84 days). The addition of MTX in the GVHD prophylaxis program seemed to decrease the incidence of acute GVHD (30% *vs* 20%), with no differences in chronic GVHD. Finally, there seemed to be a greater proportion of patients alive as well as fewer relapses at 100 days in those allogeneic transplants that had only CAMPATH + CsA/Tac conditioning than those of the MTX group (92% alive, 0 relapses *vs* 84% alive, 16% relapses).
Conclusions: These data suggest that whilst the incidence of acute GvHD may be slightly lower in the MTX group, there is also a clear prolongation of haematological recovery and inpatient stay in this group. This analysis suggests that the addition of MTX to a two drug Campath-based GvHD prophylaxis may not necessarily be as beneficial as originally intended, as it appear to confer prolonged hospitalisation and potential morbidity.

**P532**

**Low-dose thymoglobuline prevents grade II-IV acute and extensive chronic GvHD after allogeneic HSCT**

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Background: Graft versus host disease (GVHD) is a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). The addition of high-dose antithymocyte globulins (ATG) to standard GVHD prophylaxis has proved to lower the incidence and severity of chronic GVHD (cGVHD), but might increase the relapse rates of hematologic malignancies and certain type of infections.
Objective: We wanted to review the impact of low-dose ATG on short and long-term outcome on a series of allo-HSCT recipients.
Patients and Methods: We retrospectively studied 76 pts (49 male, 27 female) who underwent allo-HSCT in our institution between January 2008 and April 2011. Median age was 42 years (range: 2-65). The pts were diagnosed with acute leukemia (35.7%), MM (28.9%), NHL/HL (22.2%), MDS (7.9%), and others (5.3%). 50% of pts received allo-HSCT from HLA identical siblings, and 50% from unrelated donors. The stem cell source was peripheral blood in 56.6%, bone marrow in 32.9% and umbilical cord blood in 10.5%. 28.4% pts received reduced intensity conditioning regimens. 81,9% pts received rabbit ATG (Thymoglobulin) as a part of the conditioning, the majority of them at low doses (median 2,7 mg/Kg, range: 0-8 mg/Kg).
Results: Incidence of grade II-IV acute GVHD (aGVHD) was 34.3%. There were no significant differences between siblings and unrelated donors, but aGVHD was more frequent with female *vs* male donors (46.9% *vs* 27.3%) ($p<0.05$). Median dose of ATG was 1.85 mg/kg in pts who developed aGVHD and 2.78 mg/kg in pts who did not ($p<0.05$). Incidence of extensive cGVHD was 21.1% (34.3% if donor was female, and 13.9% if donor was male; $p<0.05$). Median dose of ATG was 1.23 mg/kg in pts who developed extensive cGVHD and 2.81 mg/kg in pts who did not ($p<0.004$). Overall survival at 3 years was 69% (follow-up: 35.5 months). Survival of pts without aGVHD was higher than those with aGVHD (85.2% *vs* 43.7%) ($p<0.05$). ATG, at the doses employed, did not increase the relapse of the malignancies or the infectious mortality.
Conclusions: Our results suggest that low-dose Thymoglobulin as a part of the conditioning regimen prevents the development of grade II-IV acute GVHD and extensive chronic GVHD with no increase in infectious mortality or relapse rates.

**P533**

**Comparison of pre-transplant thymoglobulin and post-transplant prednisolone as graft-versus-host disease prophylaxis in matched unrelated donor, peripheral blood stem cell transplantation**

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Introduction: *In-vivo* lymphocyte-depleting therapies as part of pre-transplant conditioning in peripheral blood stem cell transplantation (PBST) have been variably associated with reductions in acute (a) and chronic (c) graft versus host disease (GvHD), but at the potential risk of delayed immune reconstitution, increased viral reactivation rates and, in some series, increased disease relapse.
Methods: From our prospective allogeneic transplant database, we identified three sequential patients cohorts undergoing matched unrelated donor (MUD) PBSCT, who received GvHD prophylaxis with either Cyclosporin (C)+ short course Methotrexate (M), or C+M+ post-transplant (d+14 to d+84) Prednisolone (P), or C+M+ pre-transplant Thymoglobulin (total dose= 4.5 mg/kg)(T). Patients transplanted prior to 2000 were excluded to avoid confounding by historical patient and donor selection criteria. We compared the rates of aGVHD, cGvHD, disease relapse, CMV and EBV reactivation and overall survival (OS) of these three cohorts. aGVHD (up to day +100) and cGvHD (beyond day +100) were scored using Przepiorka 1995 and Wolff 2010 criteria respectively. CMV reactivation (all three cohorts) and EBV reactivation (CMT cohort only) were assessed by weekly PCR.
Results: A total of 133 patients were identified. Details are in Table 1.
Five year relapse free survival (RFS) and (OS) were not statistically different between the three cohorts; RFS; CM= 63%, CMP= 55%, CMT= 63%.
OS; CM=65%, CMP=56%, CMT=69%. Five year OS in high-risk *vs* low risk disease was CM=52% *vs* ND, CMP=48 *vs* 73% and CMT=65 *vs* 77%. Grade (II-IV) aGvHD rates for CM=88% (median onset=36d), CMP=69% (median onset=59d), CMT= 42% (median not reached) ($p=0.0001$). Limited and extensive cGvHD by 12m occurred in CM=90% CMP=90%, CMT=24% and by 36m CM=90%, CMP=90%, CMT=60% ($p=0.0001$). Extensive cGVHD by 12m were CM=25%, CMP=36%, CMT=12% and at 36m CM=67%, CMP=67%, CMT=21% ($p=0.0003$). CMV reactivation at any time prior to 100 days was highest in the CMP group (69%) compared to CM=48%, CMT=40%. EBV reactivation occurred in 28% of the CMT group with no cases of PTLD in any of the three cohorts.
Conclusions: The use of pre-transplant Thymoglobulin (but not post-transplant prophylactic prednisolone) substantially reduces acute and chronic GVHD incidence in recipients of MUD PBSCT without adversely affecting viral reactivation or disease relapse even in high-risk disease.

**P534**

**A comparison of three approaches to the post-transplant GvHD prophylaxis (single-centre study)**

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Background: Cyclosporin-A (CSP-A) still remains the essential immunosuppressive drug in the GVHD prophylaxis after allogeneic stem cell transplantations (SCT). The combination of CSP-A with methotrexate (MTX) or mycophenolate mofetil (MMF) can further reduce the risk of GVHD. Presented analysis retrospectively assessed the results of allogeneic SCT in regard of used three regimens of posttransplant GVHD prophylaxis - CSP-A only, CSP-A + MTX and CSP-A + MMF.
Patients and Methods: CSP-A only was administered in 56 (37%), CSP-A + MTX in 38 (26%) and CSP-A+MMF in 56 (37%) allografted patients. There were found significant differences among those groups in recipients age (median 48 *vs.* 45 *vs.* 32 years,

**[P533]**

| GVHD regimen | n | Median age (y) | RIC (%) | High risk disease (%) | Female donor to male recipient(%) | Median follow up (m) |
|---|---|---|---|---|---|---|
| CM | 24 | 45 | 3 (12) | 20 (83) | 6 (25) | 103 |
| CMP | 47 | 45 | 6 (12) | 35 (74) | 2 (4) | 94 |
| CMT | 62 | 43 | 15 (24) | 42 (68) | 5 (8) | 33 |

p=0.005), the diagnosis of acute leukemia (38% *vs.* 55% *vs.* 64%, p=0.02), disease remission at SCT (13% *vs.* 39% *vs.* 59%, p=0.000002), the use of ATG in conditioning (68% *vs.* 24% *vs.* 36%, p=0.00005), allografting from sibling (55% *vs.* 95% *vs.* 36%, p=0.0000002) and HLA-matched donor (93% *vs.* 97% *vs.* 68%, P=0.000005) and SCT after myeloablative conditioning (4% *vs.* 41% *vs.* 55%, p=0.00000001). The differences in the risk of GVHD development, disease relapse and non-relapse mortality (NRM) were assessed with the multivariate Cox proportional hazards model. Kaplan-Meier method was used to compare the probability of event-free survival (EFS) and overall survival (OS).
Results: There were found no significant differences in the risk of acute (32% *vs.* 39% *vs.* 36%, p=0.6; HR 1.18 [95%CI 0.86 - 1.62], p=0.3) or chronic GVHD development (25% *vs.* 29% *vs.* 36%, p=0.6), disease relapse (20% *vs.* 30% *vs.* 14%, p=0.2; HR 0.87 [95%CI 0.56-1.37], p=0.6), NRM (20% *vs.* 42% *vs.* 32%, p=0.1; HR 1.27 [95%CI 0.9-1.79], p=0.2), 5-year EFS (52% *vs.* 32% *vs.* 43%, p=0.1; HR 1.0 [95%CI 0.73-1.34], p=0.9) and OS (53% *vs.* 34% *vs.* 53%, p=0.25; HR 1.03 [95%CI 0.78-1.35], p=0.9).
Conclusion: There cannot be preferred any of three presented GVHD prophylaxes without respect to the primary disease and its activity, used conditioning, donor characteristics and other parameters potentially influencing the outcome of allografted patients. The intensity of immunosuppressive prophylaxis should be individually chosen upon the consideration of all prognostic factors and potential transplant related risks.
Supported by the grants MSM-6198959205 and LF-2011-006.

**P535**
**Identification of a distinct NK cell subset for GvHD prevention in adoptive cell therapy**
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Clinical studies exploiting the impact of natural killer (NK) cells during hematopoietic stem cell transplantation have provided promising results in GVHD prevention. It is known that NK cells are a heterogeneous population that can be divided into functionally distinct NK cell subpopulations. Murine NK cells can be separated along their expression of CD117 (c-kit), CD27 and CD11b. The functional relevance of the distinct NK subsets in graft-versus-host-disease (GVHD) has not been investigated in detail so far.
In the first part of this study, we optimized the isolation of NK cell subsets and characterized the genomic, phenotypic and functional profile of these different NK cell subsets.
Microarray analysis provided important insights into the distinct genomic profiles of these subsets. Functional *in vitro* analysis clearly demonstrate that CD11b+ NK cells develop the highest tumorlytic capacity (60%) whereas CD27+ CD11b- only develop about 25% at an effector-target ratio of 5:1. Interestingly, CD27+ NK cells provided the highest IFN-gamma production upon incubation with tumor cells and/or IL-2.
We further analyzed the phenotypical changes and functional capacities upon stimulation and expansion in cytokines such as IL-2 and IL-15.
Next, we addressed the role of distinct cytokine activated NK cell subpopulations in adoptive cell therapy.
The final part of our work investigates the impact and pathomechanism of the distinct preparations of either the CD27+ NK cell subset or the CD11b+ NK cell subset in the prevention of GVHD in allogeneic mismatched BMT. Our comparative study outlines that these subpopulations differ significantly in attenuation of GVHD. Further *in vitro* experiments to investigate the role of these NK subsets on mixed lymphocyte reactions indicate that the underlying pathomechanism is based on a direct lytic effect on allogeneic dendritic cells (DC). The elimination of recipient DCs *in vivo* might explain the reduced induction of alloreactive T cells and thereby the GVHD prevention.
In addition, we clearly show that NK cells need specific stimuli to gain the capacity of GVHD protection.
In sum, this preclinical study is of high importance for the further optimization of NK cell therapy in HSCT.

**P536**
**Model for experimental GvHD colitis and endoscopic evaluation: new insights into intestinal immunopathogenesis**
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Hematopoietic stem cell transplantation (HSCT) represents often the only curative option for severe forms of malignant neoplasia. However, therapeutic success of HSCT is limited by the occurrence of graft versus host disease (GVHD) as a life-threatening adverse effect.
Beside liver and skin manifestation, GVHD patients suffer frequently from a severe intestinal inflammation. Actually, there exist only unspecific therapeutic regimens for the control of gastrointestinal GVHD. An improved understanding of the exact immunopathogenesis of intestinal GVHD is needed to develop innovative therapeutic strategies.
In our study, GVHD colitis was characterized in a murine model of full allogeneic mismatch BMT following myeloablativ irradiation with an allogeneic T cell transfer. *In vivo* endoscopic imaging enabled us to monitore the course of GVHD colitis over time.
Around day 14 after BMT and T cell transfer, recipient mice started to develop first clinical symptoms and endoscopic signs of GVHD colitis. Monitoring the intestinal inflammation in the described experimental setting over time, endoscopic scores as well as histological analysis indicated a chronic course of GVHD colitis. Analysis by immunofluorescence microscopy confirmed that the observed GVHD colitis was based on a massive infiltration of congenic CD45.1+ donor lymphocytes into the colonic lamina propria. The active participation of infiltrating lymphocytes in the inflammatory process was reflected by a high expression of transcription factors like T-bet and Eomes and by a marked induction of pro-inflammatory cytokines (IFNg, IL-6, IL-17) within the colonic tissue. At the same time, increased intestinal levels of activated caspase-3 could be detected by Western blot and implicated the presence of apoptotic cells within the inflamed colon. Interestingly, apoptotic cell death was not restricted to intestinal epithelial cells, but an increasing number of TUNEL positive immune cells could be detected within the lamina propria during the course of disease.
The described GVHD model in combination with the technique of *in vivo* miniendoscopy represents an optimal and innovative experimental setting for the improved characterization of GVHD colitis. Exact analysis of the cellular and molecular mechanisms, which underlie immune cell activation and apoptosis within the lamina propria will help to identify gut specific targets for optimized therapy of GVHD colitis.

**P537**
**Tacrolimus versus cyclosporine A in prophylaxis of acute graft-versus-host disease after allogeneic haematopoietic stem cell transplantation in patients with acute leukaemia**
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Background: Acute graft-vs.-host disease (aGvHD) still remains one of the major obstacles to successful allogeneic hematopoietic stem cell transplantation (allo-HSCT). The ability to prevent aGvHD, i.e., the application of successful prophylaxis, is

the cornerstone of success. Immunosupression with pharmacological agents such as cyclosporine (CsA) or tacrolimus (Tx) is more often use for aGvHD prophylaxis.
Patients and Methods: From 2000 till 2009 115 patients (pts) with acute leukemia (AML-61 pts, ALL-54 pts, complete remission(CR)/relapse – 61%/39%) underwent allo-HSCT from related (n=52, 27%) and unrelated donors (n=63, 73%). Median age – 43 (14-66), male/female – 66/49. Acute GvHD prophylaxis were CsA-based regimen – 80 pts, Tx-based – 35 pts. Transplant characteristic were similar in both groups.
Results: The incidence of acute GvHD II-IV grades was significantly lower in patients who received Tx *vs* CsA (29% *vs* 54% in related allo-HSCT, p<0,05, 32% *vs* 60% in unrelated allo-HSCT, p<0,05). 3-years OS after allo-HSCT in relapse was significantly higher in Tx group (44%) *vs* CsA (15%) (p<0,05). But there was no significant difference in 3-years OS after allo-HSCT in CR between CsA *vs* Tx group – 44% *vs* 45%, respectively. Allo-HSCT in relapse with MAC was result in 43% 3-years OS *vs* 23%, Tx *vs* CsA groups, respectively (p=0.09). 3-years OS after allo-HSCT in relapse with RIC was also higher in Tx group (41%) *vs* CsA (13%) (p=0,08).
The incidence of infections was similar in two study groups and didn't depend on disease stage and conditioning regimens. Infections were cause of mortality in 10 pts (16,7%), aGvHD in 15 pts (26,8%), relapse in 16 pts (27%), multisystem organ failure in 10 pts (17,7%).
The toxicity profiles of CsA and Tx were similar. But the differences were in the incidence of hypertension – higher in CsA group (38% *vs.* 24%, p=0,04), but neurological complications (headache, tremor, paresthesia) and nephrology complication were higher in Tx group (44% *vs.* 30%, p=0,02 and 21% *vs* 14%, p=0,05, respectively).
Conclusion: Use of Tx is preferable in high risk of aGvHD group because it was superior for aGvHD prophylaxis and in relapse pts at allo-HSCT because of better OS. But Tx has more toxic complications such as neurological and nephrology disturbances whereas hypertension is more commonly seen with CsA.

**P538**
**Prevention of acute GvHD by sustaining induction of tryptophan catabolism**
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Reducing or preventing intestinal graft-versus-host disease (GVHD) without a wide systemic suppression would lead to preserve the beneficial graft-versus-tumor (GVT) effect of donor T cells resulting in significantly improve overall survival in patients who undergo allogeneic hematopoietic stem cell plantation (HSCT). Indoleamine 2,3-dioxygenase (IDO) is an intracellular enzyme that catalyzes the first and rate-limiting step in tryptophan catabolism. Induction of IDO expression can effectively suppress the local inflammatory response by depletion of tryptophan and/or accumulation of catabolites known as kynurenines. Recently, it has reported that IDO expression is induced in the colon after allogeneic HSCT and it caused local inflammation decreases and reduces GVHD severity. These results indicate that modulation of IDO in GVHD target organs may represent an interesting strategy for limiting GVHD. However, the physiological mechanism of this phenomenon remains unclear. We found that IDO-mediated tryptophan catabolism induced by donor T cell-derived IFN-gamma is transient and incomplete in colon of recipient mice after allo-HSCT, which influence little suppressive activity on GVHD severity and lethality. Administration of kynurenines (3-hydroxykynurnine+ 3-hydroxyanthranilic acid) during the IDO expression time period prevents GVHD lethality, which was caused by promoting effector donor T cell death in colon tissue. Our data suggest that strategies to sustain the IDO-mediated tryptophan catabolism in GVHD target organs decrease GVHD severity without impairment of GVT effects.

**P539**
**Patient compliance with oral therapies after allogeneic stem cell transplant: is it optimal?**
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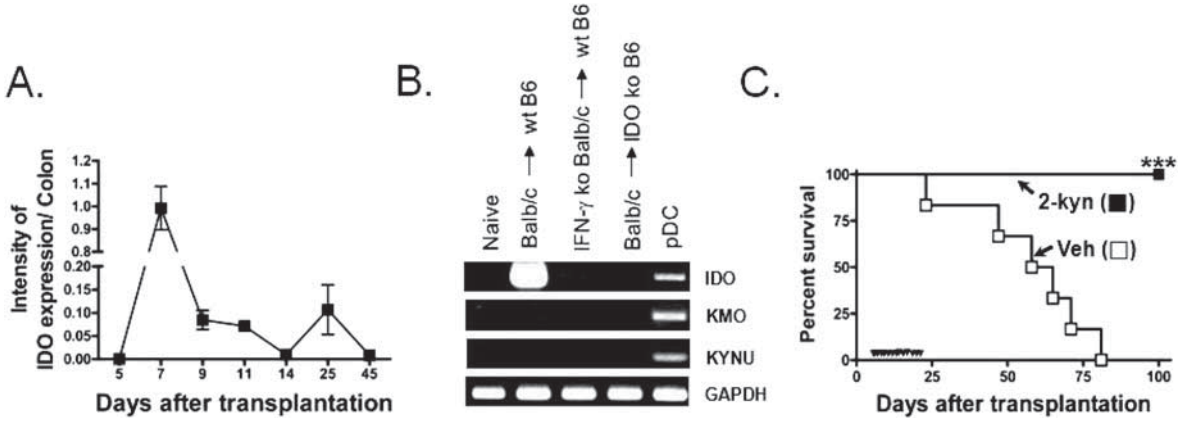
Allogeneic stem cell transplant (SCT) recipients need to take many oral medications, mainly for prophylaxis or treatment of graft-versus-host disease (GVHD) or infections. Although patients are supposed to have been informed of the rationale of each of their treatments, and have received practical guidelines for timing and modalities of intakes, they may be poorly observant or compliant, especially if they do not know about the real importance of the medications.
Objectives: In order to assess the level of knowledge of our SCT recipients about their oral drugs, and their observance of treatment, we designed a questionnaire. The primary objective was to have baseline data in order to implement a program of therapeutic education within our SCT unit.
Methods: Our questionnaire covered items on indication, possible side effects, precautions, intake modalities, timing, and observance of treatment. It was focused on 6 main oral therapies: cyclosporine, steroids, mycophenolate mofetil, trimethoprim-sulfamethoxazole, penicillin, and posaconazole. The questionnaire was run by a pharmacy student, not directly involved in the transplant unit, to the patients, and eventually their relatives if present at the consultation.

**[P538]**



Results: 24 patients (M/F: 10/14), aged 20-70 years, were included. For all the drugs, the patients were asked about a given drug only if they were receiving it, or if they previously received it. The overall results showed that > 65% of the patients knows the indication of the drugs, and > 50% knows the number of daily intakes. Only 86% of the patients know about the need for salt-free diet with steroids, and 60% about the risk of diabetes. Almost all patients know the rationale for cyclosporine in GVHD, but half of them say they have or had difficulties to take it, and 21% say they regularly missed doses. According to the drug, only 50 to 85% of the patients declared to have received enough information from the staff about oral medications.
Conclusion: Although information is given to the patients before and after SCT, this information should be better formalized at different levels, by transplant physicians and nurses, in order to improve the understanding of their treatments, and consequently their observance. A better observance should translate in a better efficacy.

**P540**
**Tailoring the GvHD prophylaxis regimen according to transplantation-associated toxicities – replacing the day 6 dose of methotrexate with mycophenolate mofetil**
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Background: Previous studies showed that graft *vs.* host disease (GVHD) prophylaxis with cyclosporine and mycophenolate mofetil (MMF) compared to cyclosporine and methotrexate (MTX) is associated with a decreased incidence of mucositis while transplantation outcome remains unchanged.
Objective: To show the effect of replacing the third dose of methotrexate with the initiation of MMF in patients with early post transplantation toxicities.
Methods: We prospectively collected data from all consecutive patients who were given allografts from 10/10 HLA identical related and unrelated donors between the years 2007 and 2011. Patients were assessed daily for transplantation associated toxicities. Patients who did not have significant evidence for toxicities (MTX group) were given 3 doses of MTX. For patients with significant toxicities, the day 6 methotrexate dose was eliminated and treatment with MMF was initiated (MMF group).
Results: The MTX group, (n=33) and the MMF group (n=31) were comparable in donor source, percentage of male recipient with female donor, conditioning intensity, and status of disease. Median age was statistically significant higher in the MTX group (53 *vs.* 44 years, p=.02) and more patients in this group were given reduced toxicity myeloablative conditioning (p=.03). Median follow up was 13 (range, 2-50) months. Fewer patients in the MMF group had an elevated bilirubin levels (p=.04), however there was no difference in the incidence of sinusoidal obstruction syndrome (p=.8). There was no difference in the percentage of patients with grade 3-4 mucositis (p=.4) or in the percentage of patients receiving IV morphine for pain control (p=.7). The median period of neutropenia was shorter in the MMF group (9 *vs.* 11 days, p=.03). There was a higher incidence of overall acute GVHD in the MMF group compared to the MTX group (85% *vs* 44%, p<.01) however the incidence of grade 3-4 acute GVHD was similar (14.2% *vs.* 13.8%, p=.81, respectively), Figure. There was no difference between the two groups in the incidence of extensive chronic GVHD, relapse rate, non relapse mortality or overall survival.
Conclusions: In this pilot study, tailoring of the GVHD prophylaxis regimen based on day 6 documented toxicities seemed to decrease the early post transplantation complications, however a higher incidence of non-severe acute GVHD was observed. A randomized controlled trial to evaluate this approach is warranted.

**P541**
**Treatment of steroid resistant grade II to IV acute GvHD by infusion of mesenchymal stroma cells expanded with human plasma and platelet lysate – a phase I/II study**
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Introduction: A life-threatening complication of HSCT is aGVHD and reduces substantially efficacy of HSCT. The outcome of patients with severe steroid-resistant aGVHD is very poor. Therefore, it remains important to search for new therapeutic strategies for the treatment of aGVHD.
Objective: Feasibility and efficacy of generation of MSCs expanded with human plasma and platelet lysate was tested as well as the feasibility and safety of infusion. Immunological changes after infusion of MSCs were characterized, regarding the distribution of subpopulations of immune cells, and level of cytokines in serum.
Method: In an open-label, non-randomized prospective phase I/II study MSCs extracted from bone marrow of healthy volunteers, patients with steroid-refractory aGVHD grade II-IV were treated with ~2x10$^6$/kg MSCs. For one year response rate, TRM, and adverse events were assessed, blood and serum were collected and analyzed by flowcytometry and luminex.

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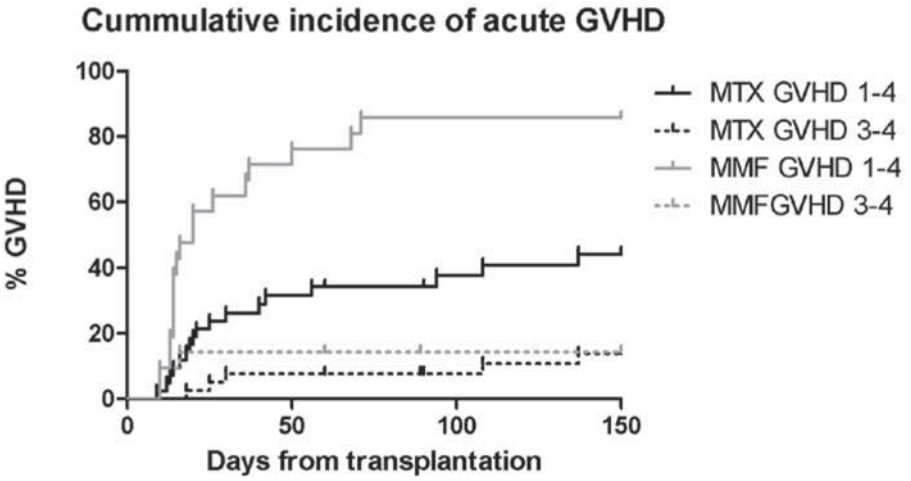

Results: From 2009-2010, 18 patient were available for analysis, 5 children and 13 adults. Median age was 32.5 years (1.3-65.9). Organs involved in aGVHD: skin (67%), GI-tract(83%) and liver(28%). Overall grade was II(22%), III(72%), and IV(6%). Median follow-up was 5.5 months (0.33-12). CR overall was observed in 11(61%) after a median of 65 days (10-184). The OS was significantly better in responders when compared to non-responders (p <0.001). Of the 11 patients who reached a CR, 8 patients relapsed approximately 2 months after reaching CR (median 59 days, range: 1-244). Three children relapsed with clinical signs of an allo-immune-lung, auto-immune-cytopenia or limited cGVHD and all 5 adults relapsed with GVHD of gut (median 98 days after reaching CR, (35-302)). However, GVHD of the gut was then again sensitive to steroids. Overall, 7 patients died, 4 due to progression of aGVHD, 1 due to abdominal bleeding and 2 due to sepsis. Immunological patterns which associate with clinical response were also detected.
Conclusion: MSCs are feasible, safe and very effective in steroid-resistant aGVHD grade II-IV. An immunological pattern of the patient predicts the clinical response.

**P542**
**Treatment of severe graft-versus-host disease with mesenchymal stromal cells: a comparison with non-MSC treated patients**
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In this study we evaluate the use of mesenchymal stromal cells (MSC) as treatment for severe acute graft versus host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT) in adults. The primary endpoint was death by any cause. Between October 2001 and August 2006, 15 patients received MSC as treatment for grade III (n=11) or grade IV (n=4) acute GVHD. The control patient group consisted of all adult patients with severe steroid resistant acute GVHD during the same period of time who were not given MSC (n=13). There were no significant differences in patient and donor characteristics between these two groups.
The overall survival 100 days, one and two years after GVHD diagnosis was 53%, 20% and 7% for the study patients and 38%, 15% and 8% for the controls (p=0.57). Median survival after GVHD diagnosis was 105 days (18-1856) and 49 days (6-2107) (p=0.21) for the study and control group, respectively. No effect of number of MSC doses was found. Invasive fungal infection (IFI) was more common in MSC treated patients, 67% *vs.* 23%, p=0.02.
We speculate that MSC-induced indoleamine-2,3-dioxygenase and kynurenine may over-activate the inflammatory response to fungi, paving the way for IFI.
In conclusion, we found that treatment of severe GVHD with MSC showed no advantage in survival compared to conventional treatment and a high risk for IFI. Ongoing randomized studies will hopefully shed light on this finding.

**P543**
**Modelling the effects of acute graft-versus-host disease on recipient bone-marrow mesenchymal stromal cells**
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Objective: Recently the bone marrow stroma gained importance in the immunopathologic concept of acute Graft-versus-Host-Disease (aGvHD). In this study we focused on the effect of soluble inflammatory mediators and their antagonists on Mesenchymal Stromal Cell (MSC) functions.
Methods: We cultured primary human MSC with conditioned medium containing 50% mixed lymphocyte reaction (MLR) supernatant. In some experiments TGF-beta and IL10 was added. With these primed MSCs co cultures with primary human

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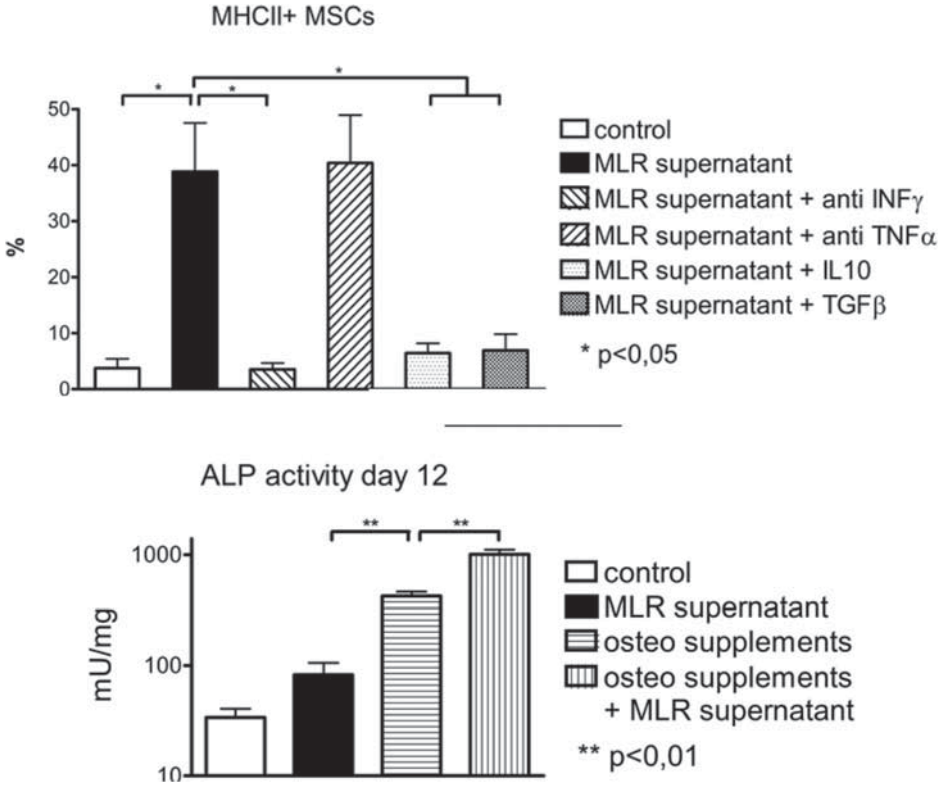

CD34+ hematopoietic stem and progenitor cells (HSPCs) were performed.
Results: In line with previous reports, we confirmed that INF-gamma up regulates MHCII on MSCs. This up regulation could be reduced 2 folds through TGF-beta and IL10 supplement (Figure 1). The 2 to 3 folds increased proliferation could be antagonized by adding TGF-beta. Osteogenic differentiation was induced by MLR supernatant treatment alone. When osteogenic differentiation supplements were added the Alkaline Phosphatase (ALP) activity increased two folds (figure 2). In the three week co culture both the number of Colony Area Forming Cells (CAFC) counted and the number of Colony Forming Units (CFU) observed in the following CFU- Granulocytes, Erythrocytes, Monocytes, Macrophages assay (CFU-GEMM) was reduced.
Conclusion: Our data suggest that a pro inflammatory stimulus changes several basic MSC properties. MSCs lose their immunoprivileged status and acquire Antigen Presenting Cell (APC) functions. Regarding their tissue regenerative potential MSCs enhanced proliferation and osteogenic potential might lead to a tissue imbalance in the bone marrow niche. The reduced capacity of MSC to support HSPC could be one explanation for the fact that patients with aGvHD suffer from hematopoietic insufficiency and subsequently have a predisposition for infectious complications. Simulating an anti-inflammatory response these changes could be partially reversed. This observation is in line with the cytokine storm concept which hypothesizes that the imbalance between pro and anti-inflammatory factors is crucial for the maintenance of aGvHD.

**P544**

**Results for the treatment of a-GvHD in second, third and fourth line in a single centre**

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Objectives: Acute graft versus host disease (aGVHD) is the main cause of morbi-mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Standar treatment for the first line are steroids that can be effective in about a 50% of patients. Nonetheless another 50% of patients would need second or more lines of treatment for its control. Our goal is to analyse the results for the second or more lines of treatment in our institution.
Patients and Methods: Between december 1980 and december 2010 we carried out 712 allo-HSCT (564 related; 147 unrelated). We analyse the results using 4 lines of treatment: corticoids ≥ 2 mg/kg/day, ATG, anti-CD25 and anti-TNF.
Results: 423 patients (59%) received steroid treatment (≥ 2 mg/kg/day): 369 patients (87,2%) obtained a complete response, 26 patients didn´t respond (6,1%) to steroids and 27 patients died (6,4%) due to aGVHD associated with another causes (mainly infections).
Out of 369 patients that obtained a complete remission after first line of treatment, 139 (37,6%) showed an aGVHD reactivation and 20 patients among them (14,3%) didn´t respond to a second course of steroids.
In sumary, 46 patients (10,6%) received a second line treatment due to corticoids-resistant aGVHD. Following the literature, this condition was defined as progression in 1 grade in three days, failure in obtaining 1 grade of improvement after 5-7 days or imcomplete response after 14 days of treatment with steroids to ≥ 2 mg/kg/day.
As second line treatment, micophenolate mofetil was added to ciclosporine or tacrolimus (associated to steroids) in 7 patients, obtaining 4 complete responses (57%), 27 patients received ATG (6 complete responses; 23%) and 12 patients received anti-CD25 MnAb (7 complete responses; 58,3%).
Eleven patients (2,5%) received third line treatment: 8 patients were treated with anti-CD25 (2 complete response; 25%), 2 were treated with ATG (no response) and 1 with anti-TNF(no response).
Only one patient received fourth line treatment with anti-TNF without response.
Conclusions: In our experience a 10,9% of patients with aGVHD needed a second line treatment, and only a 41,3 % of these patients (19/46) responded. We obtained the best results in second and third line with anti-CD25. We didn´t see responses with ATG or anti-TNF in third line in our patients.

**P545**

**Extracorporeal photopheresis: effective second-line therapy for steroid-dependent and refractory acute graft-versus-host disease**

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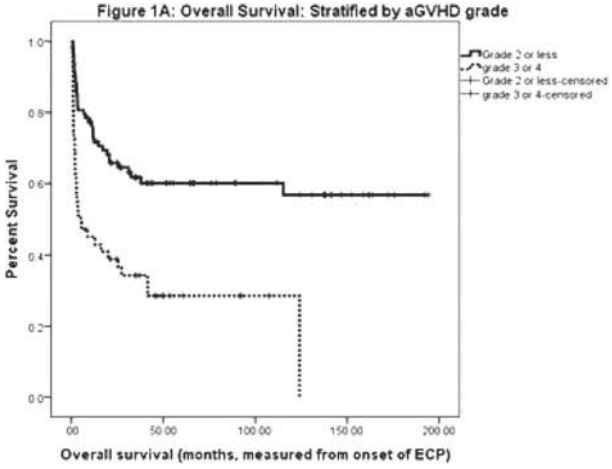
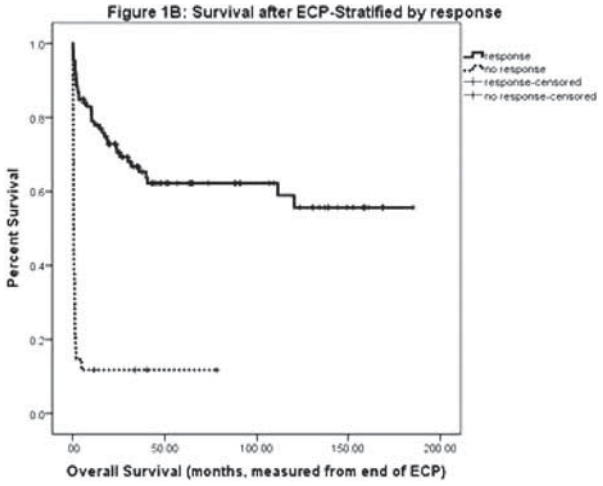
We studied the efficacy of extracorporeal photopheresis (ECP) used as second line therapy in patients (pts) with steroid refractory (SR) (progression after 3 days (d) or no response after 7 d) or dependent (SD) (recurrence during steroid taper) acute graft-versus-host disease (aGVHD) at 3 centers. 144 pts (Austria-92, USA-29; UK-23) were treated with ECP (SD -50, 35%; SR-89, 62%; missing-5) at a median of 42 (range 12-99) d after transplant. At ECP-onset, stage 3-4 aGVHD organ involvement included: skin (29, 20%), GI (58, 40%), and liver (26, 18%) and grade (gr) 3-4 aGVHD was present in 51 (35%) pts. All pts received steroids prior to ECP at 1 mg/kg (35, 24%), 2 mg/kg (101, 70%) or > 2 mg/kg (6, 4%) prednisone equivalent for a median of 17 (range 10-91) d. Median number of ECP treatments was 12 (range 2-90) and 107 (74%) pts responded (CR- 64%; PR-10%). Gr 3-4 aGVHD was present in 25 pts (17%) at end of ECP. The mean steroid dose at ECP-onset and end of ECP was 1.8 mg/kg (range 0-10) and 0.6 mg/kg (range 0-4) (P=0.008) with responders having a lower dose at end of ECP (responders, 0.1 (range 0-2) mg/kg; non-responders 1.9 (range 0.15-4) mg/kg; P<0.001). ECP response was superior after ablative regimen (MAC) compared with other regimens (79% *vs.* 63%, P=0.044). Pts with aGVHD ≤ gr2 (86% *vs.* 53%, P<0.001) and GI stage ≤2 at ECP-onset (84% *vs.* 60%, P=0.002) had a higher response rate. Adjusted for regimen-intensity and GI stage, aGHVD gr at ECP-onset was a predictor of response (OR=3.81, 95% CI 1.58-9.1 P=0.003). The median follow-up after ECP-onset is 21.7 (range, 0.4-194) months (m) and 2-yr survival is 56%.
Causes of death include: GVHD-36, relapse-17, infections-13, other-6. Median survival after ECP-onset was superior for pts who had a MAC (not reached (NR) *vs.* 6.8 m, P=0.012), and aGVHD ≤ gr2 at ECP-onset (NR *vs.* 5.1 m P<0.001) (Figure 1A). Pts with ECP response had a superior survival (from end of ECP) compared to non-responders (NR *vs.* 0.5 m P<0.001) (Figure 1B) with a 69% 2-yr survival in ECP-responders. In multivariate analyses, lack of ECP response was a predictor of inferior survival (HR=7.85, 95% CI 4.5-13.65 P<0.001), adjusted for regimen-intensity (other regimens-HR=1.76, 95% CI 1.03-3.01 P=0.036) and aGVHD gr at onset (>gr 2-HR=1.75, 95% CI 1.04-2.95 P=0.035). ECP is an effective steroid-sparing salvage therapy for pts with SD or SR aGVHD. A prospective randomized study of ECP versus other agents in pts with SD or SR aGVHD is warranted.




**P546**

**The first prospective study of extracorporeal photochemotherapy in children with refractory acute graft-versus-host disease after allogeneic stem cell transplantation: preliminary results**

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We report the preliminary results of the 10 first patients included in a prospective multicentric study assessing extracorporeal photopheresis (ECP) for second or third-line therapy in children with steroid refractory aGvHD.

Methods: 3 ECP sessions a week during 2 weeks, then: 1/ patients in complete response (CR) receive 1 session a week until steroids are tapered to 0.5 mg/kg/d then stop; 2/patients in partial response (PR) receive 2 sessions during 2 weeks then tapering until steroids are tapered to 0.5 mg/kg/d then stop; 3/in case of absence of response (NR) then the patients are switched off the protocol. Final evaluation at week 10. Refractoriness to corticosteroids was defined as progression after at least 48 hours steroids at 2 mg/kg/d or stable disease or progression after 4 days steroids at 2 mg/kg/d or stable disease or progression after 2nd line therapy during at least 8 days. CR was defined by the resolution of all signs of aGvHD, PR by improvement of at least one IBMTR grade and NR by the absence of improvement.

Results: 10 patients aged 3 to 17 yrs. The indications of transplantation were: acute leukaemia (n=5), 3 aplastic anemia (n=3), non-Hodgkin's lymphoma (n=1) and solid tumor (n=1). The donor was a sibling HLA-identical donor (n=2), an unrelated HLA-identical donor (n=3), and a mismatch unrelated donor (n=5). The stem cell source was cord blood (n=4), bone marrow (n=3) and peripheral stem cell (n=3). Five patients had received a second-line therapy with anti-TNF biologics and/or anti-CD25.

At the beginning of ECP, 3 of 10 pts suffered from aGvHD grade 1-2, 3 pts from grade 3 and 4 pts from grade 4. The median duration from onset of aGvHD until start of ECP was 34 days (range 8-212 days). The median number of sessions was 13 (range 5-27). ECP was well tolerated, no severe side-effect occurred. Two patients died before 2 weeks ECP, while non-responders (grade IV). The median follow-up after ECP was 19 months (range 1.4-30 months). Seven patients achieved a CR, 1 pt a PR (grade IV). Overall, 4 pts died at a median delay of 48 days after the beginning of ECP (range 18-244 days). One from multi-organ failure, two from adenovirus infection, and one from chronic GvHD. All had grade IV aGvHD. The 1-year survival probability is 53%.

(preliminary) conclusion: The study is ongoing; the outcome is excellent for patients with grade I to III aGvHD.

**P547**

**Extracorporeal photopheresis in 74 patients with acute and chronic GvHD: comparison of the ECP techniques COBE® Spectra and Therakos UVAR® system**

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Extracorporeal photopheresis (ECP) is an important treatment for steroid-refractory acute and chronic graft-versus-host disease (aGVHD and cGVHD). Within a retrospective analysis the results of treatment with ECP in aGVHD (n=35; grade 2: n=15, grade 3: n=16, grade 4: n=4) and cGVHD (n=39; moderate: n=16, severe: n=22, severe overlap-syndrome: n=1) were evaluated. 62 patients were treated with ECP employing the COBE® Spectra LRSTM System, and 12 patients obtained the procedures with the Therakos UVAR® XTSTM System.

Results: No significant difference in response rates and overall survival could be shown in patients treated either with COBE® or Therakos System. A complete remission (CR) and a partial remission (PR) was observed in 9 (25.7%) and 5 (14.3%) patients with aGVHD and in 5 (13.2%) and 11 (28.9%) patients with cGVHD without application of any new additional immunosuppressive therapy (IST) during ECP. In 9 additional patients with aGVHD a CR or PR was achieved with additional IST started during ECP interfering with evaluation of response. These patients were classified as "treatment failure" (TF). Four additional patients with cGVHD classified as TF achieved CR or PR with additional IST initiated during ECP.

In aGVHD 22 (63%) patients and in cGVHD 21 (55%) patients could taper steroids ≥ 50%. In 9 (26%) patients with aGVHD a reduction of 1 immunosuppressive medication, in 7 (20%) a reduction of 2 medications and in 3 (9%) a reduction of 3 medications was achieved. In 11 (29%) patients with cGVHD a reduction of one immunosuppressive drug and in 4 (10.5%) a reduction of two immunosuppressive medications was possible.

Treatment related mortality (TRM) was observed in 16 (46%) patients with aGVHD (causes of death: infection (n=7), progressive GVHD (n=8), other (n=1) and 12 (32%) patients with cGVHD (causes of death: infection (n=6), progressive GVHD (n=2), other (n=4). At last follow-up, 12 (34.3%) of patients with aGVHD and 23 (58.9%) of patients with cGVHD were alive. Patients with aGVHD achieving a CR or PR showed

a significant improved overall survival (OS) (median OS: 43 months, range: 5–118) than nonresponder (median OS: 25 months, range: 2.5–51) (p =.014).
Conclusion: ECP is associated with significant response rates and allows a successful reduction of steroids and IST in patients with steroid-dependent and -refractory aGVHD and cGVHD. Response to ECP is associated with a significant improved overall survival in aGVHD.

**P548**

**Extracorporeal photopheresis in patients with high bleeding risks**

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For anticoagulation in extracorporeal photopheresis (ECP) to prevent clotting in the extracorporeal circuit, the manufacturer's recommendation is the use of heparin (normally 10.000 Units). However, patient with acute graft-versus-host disease (aGvHD) after allogeneic stem cell transplantation are at high risk for bleeding complication due to low platelet counts, intestinal lesions from aGvHD or impaired hepatic function. For these patients, alternative anticoagulation using acid citrate dextrose (ACD-A) has been infrequently used in small patient cohorts.
We investigated the safety and efficacy of this approach in 94 consecutive patients (43 male, 51 female) with aGvHD (45% with GvHD of the intestine) undergoing ECP with ACD-A anticoagulation at a single institution 2 to 3 times per week on a weekly basis until complete resolution of aGvHD. A total of 1242 ECP procedures were analyzed with respect to side effects and changes in haemoglobin and platelet levels. Moreover, in a proportion of ECP treatments activated partial thromboplastin time prothrombin time (aPTT) was monitored. Priming of the separator was performed with 1.000 Units of heparin followed by anticoagulation with ACD-A at a ratio of 1:10 during the procedure.
ECP was tolerated well by all patients. In only 0.2 % of procedures mild citrate reactions seen as transient paresthesias were observed but resolved without the need for calcium substitution. In no case bleeding complications were noted during citrate anticoagulation. During ECP, aPTT levels increased marginally from a median of 32.1 seconds (sec; range, 25.3 to 44.5 sec) before to a median of 35.4 sec (range, 25.4 to 54.7 sec) after ECP, respectively. At start of ECP 51% of patients had platelet counts <100 G/l and 26% <50 G/l. Haemoglobin and platelet counts decreased by 11% and 14%, respectively.
In conclusion, citrate anticoagulation during ECP is a feasible and safe alternative for patients with high risk for bleeding complications, especially for those with aGvHD of the intestine and low platelet counts.

**P549**

**Extracorporeal photochemotherapy treatment of steroid refractory or hyperacute graft-versus-host disease after allogeneic haematopoietic stem cell transplantation from matched unrelated donors**

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The allogeneic hemopoietic stem cell transplantation (HSCT) has become a major therapeutic option for patients (pts) suffering in different hematologic diseases. Acute graft versus host disease (aGVHD), the leading cause of non-relapse mortality, has been observed in up to 80 % of matched unrelated (MUD) HSCT in spite of GVHD prophylaxis.
This is a retrospective analysis of extracorporeal photochemotherapy (ECP) treatment in aGVHD after MUD-HSCT (matched: 4, mismatched: 13) in our department between 01.01.2006 and 30.11.2011. 17 out of 133 MUD-HSCT pts (female: 8, male: 9, median age: 42 years) were treated by ECP because of steroid refractory (5) or hyperacute (12) aGVHD. Transplant characteristics: diagnoses: acute/chronic leukemia: 12, lymphoproliferative disease: 5, conditioning: myeloablative: 14, reduced intensity: 3; stem cell source: bone marrow: 3, peripheral blood stem cells: 14; GVHD prophylaxis: different combinations of tacrolimus, sirolimus, mycophenolate mofetil and cyclosporine A; T cell depletion: *in vitro*: 5, *in vivo*: 1.
The median time of engraftment was day +13.. The median onset of aGVHD was day +12. Skin involvement (stage: II:4, III:11, IV:2) was detected in all cases, liver involvement in 7 (stage: I:1, II:1, III:3, IV:2) and gut in 6 pts (stage: I:1, II:4, III:1) with overall Glucksberg grade (gr) I-II in 10 and gr. III-IV in 7 pts. The maximal median dose of methylprednisolone before starting ECP was 5 mg/kgbw. The median day of starting ECP was day +26. The median number of ECPs was 19. The initial ECP frequency was 1 cycle/week. The ECP was very well tolerated without major side effects. 14 out of 17 skin aGVHD (CR:10, PR:4), 2 out of 7 liver aGVHD (PR only) and 1 out of 6 gut aGVHD (PR only) responded to ECP. 7 out of 17 pts are alive. The median time between onset of aGVHD and starting ECP was shorter in the surviving group (9 days *vs* 14 days). 7 out of 10 pts with overall gr. I-II responded to ECP, but all the 7 pts with overall gr. III-IV died. However, 6 pts of the latter group received anti-GVHD monoclonal antibody therapy prior to or concomitantly with ECP treatment, which could influence its effectiveness negatively. The median follow up is 142 days following transplants.
In our series ECP seemed to be effective in aGVHD in Glucksberg gr. I-II pts. In more advanced GVHD ECP proved to be ineffective, at least together with monoclonal antibody therapy of GVHD. Delay in starting ECP worsened the outcome. Further studies are warranted.

**P550**

**Lymphocyte subpopulations analysis of extracorporeal photopheresis products in graft-versus-host disease treatment**

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Introduction: Graft versus host disease (GVHD) is the leading cause of morbidity of allogeneic stem cell transplantation (SCT). The mainstay of treatment is the use of steroids, but responses tend to be lower than 30%. Extracorporeal Photopheresis (ECP) is a useful immunomodulatory therapy in treatment of steroid refractory GVHD. However, the underlaying mechanism is not yet understood and there is little information about lymphocyte subpopulations in infused products.
Objective: To analyze the lymphocyte subpopulation infused in ECP and its clinical relevance.
Patients and Methods: 4 patients with steroid refractory GVHD who underwent ECP are studied. A Cobe Spectra (Caridian-BCT®) device was used for lymphopheresis. 1-2 patient´s volemias and target hematocrit <5% per lymphoapheresis was processed. 3 ml of 8-methoxy-psoralene aqueous solution (8-MOP) was added to a final concentration of drug of 200 ng/ml before the photo-inactivation. Each cycle (C) of ECP was conducted on 2 consecutive days/week and then every 15 days according to clinical response. Studies of lymphocyte subpopulations (T, B, NK) of the infused products were analyzed with multiparametric 4 colour flow cytometry (FC-500® Coulter). Baseline characteristic of the patients are presented in Table 1.
Results: Case 1: 1ECP-C/week x 4 and then 1C/15 days x 2 (12 infusion products) were performed with complete response (CR) after 3rd C. Case 2: 1ECP-C/week x 4 were needed with CR. Because of the good response he later received 1C/15 days x 7 (22 infusion products). Case 3: 1ECP-C/15 days initially and then 1C/week x 2 was needed with CR (6 infusion

**[P550]**

| Table 1 | CASE 1 | CASE 2 | CASE 3 | CASE 4 |
|---|---|---|---|---|
| Age | 34 | 55 | 51 | 56 |
| Gender (M/F) | M | M | F | F |
| Diagnosis | MDS | MM | AML | AML |
| Transplant | MUD-MA | Haplo-RIC | MUD-MA | MUD-RIC |
| GVHD (acute/chronic) (day/month started) | acute (day +90) | acute (day +40) | acute (day +50) | chronic (month +12) |
| Organ affected | digestive | digestive/cutaneous | cutaneous | lung |
| Grade GVHD | III | I/II | III | Severe |
| Therapy prior ECP | Steroid/MMF | Steroid/MMF/MSC | Steroid/MMF/ATG | Steroid/MMF |

MDS: mielodisplastic syndrome
MM: multiple myeloma
AML: acute myeloid leukemia
MUD: matched unrelated donor
MA: myeloablative
RIC: reduced intensity conditioning
MMF: mycophenolate mofetil
MSC: mesenchymal stem cells
ATG: antithymocyte globuline

| Table 2: Product contained cells (N=46) | CASE 1 | | CASE 2 | | CASE 3 | | CASE 4 | |
|---|---|---|---|---|---|---|---|---|
| **Total CD3 (x10^8)/ CD3 (x10^8)/kg** | | | | | | | | |
| Median | 14.6 | 0,19 | 18.4 | 0,22 | 39.6 | 0,72 | 7.3 | 0,18 |
| Range | 4.6-56.7 | 0,06-0,76 | 4.4-29 | 0,05-0,34 | 0.006-57.5 | 0,0001-1,05 | 3.8-17.4 | 0,1-0,4 |
| **Total CD4 (x10^8)/ CD3 (x10^8)/kg** | | | | | | | | |
| Median | 1 | 0,013 | 4.9 | 0,06 | 2.3 | 0,04 | 3.6 | 0,09 |
| Range | 0.6-6.3 | 0,008-0,08 | 1.1-7.7 | 0,01-0,09 | 0-2.8 | 0-0,05 | 1.8-8.5 | 0,05-0,2 |
| **Total CD8 (x10^8)/ CD3 (x10^8)/kg** | | | | | | | | |
| Median | 11.5 | 0,15 | 13.2 | 0,16 | 37.1 | 0,67 | 3.6 | 0,09 |
| Range | 3.6-50.5 | 0,05-0,67 | 3.3-22.2 | 0,04-0,26 | 0.006-54.5 | 0,0001-0,99 | 2-8.3 | 0,05-0,08 |
| **Total CD19 (x10^8)/ CD3 (x10^8)/kg** | | | | | | | | |
| Median | 5.6 | 0,07 | 0.03 | 0,0004 | 0.01 | 0,0002 | 9.6 | 0,24 |
| Range | 0.7-14 | 0,009-0,19 | 0.009-0.1 | 0,0001-0,001 | 0-0.02 | 0-0,0004 | 2.4-14.7 | 0,06-0,37 |
| **Total CD56 (x10^8)/ CD3 (x10^8)/kg** | | | | | | | | |
| Median | 1.6 | 0,02 | 1.7 | 0,02 | 2.4 | 0,04 | 1.9 | 0,05 |
| Range | 0.2-3.1 | 0,003-0,04 | 0.4-4.4 | 0,005-0,05 | 1-3.2 | 0,02-0,06 | 0.4-2.5 | 0,01-0,06 |

products). Case 4: 1ECP-C/15 days x 3 (6 infusion products) were performed with no response. A total of 46 infused products were analyzed. The analysis of the lymphocyte dose in the infused products of each patient is shown in Table 2. The products contain grate variability in lymphocyte subpopulations with predominant levels of CD3+ cells, CD4/CD8 cocient inverted, homogeneous NK subpopulation and low levels of CD19+.
Conclusions: Our results show a majority of T subpopulation and more specifically CD8 cells in infused products. Further studies to clarify the possible involvement of these populations in the pathophysiological mechanism are warranted.

**P551**

**Extracorporeal photopheresis in combined immunosuppressive therapy of steroid-refractory acute graft-versus-host disease**

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Objective: Evaluation of extracorporeal photopheresis (ECP) in combined immunosuppressive therapy (IST) of steroid-refractory acute graft-versus-host disease (aGVHD).
Methods: We performed ECP in 21 patients with steroid-refractory aGVHD after allogeneic hematopoietic stem cell transplantation (grade II (n=9), III (n=5) or IV (n=7)). HLA-matched grafts were in 13 cases, HLA-mismatched graft (1 mismatch) in 1 patient and 7 patients with haploidentical grafts. All patients received ECP as a component of combined IST. Combinations of calcineurin inhibitors/steroids/ECP were used in 6 patients, calcineurin inhibitors/steroids/MMF/ECP in 3 patients, calcineurin inhibitors/steroids/monoclonal antibodies/ECP in 11 patients and calcineurin inhibitors/steroids/monoclonal antibodies/mesenchymal stem cells/ECP in 1 patient. The median duration of ECP was 2 months and the median number of ECP cycles was 6 (range 2-24). The schedule of ECP was individual ranging from 1 to 2 procedures per week. The assessment of the effectiveness of combined IST with ECP was based on clinical manifestations of aGVHD, the change in the dose of steroids during ECP and the requirement for additional immunosuppressive therapy for aGVHD.
Results. The average aGVHD grade was 2.9 at the onset of ECP and declined to 1.95 at the discontinuation of ECP (p=0.013). At the end of ECP course 48% of patients had clinically insignificant aGVHD grades 0-I. The average dose of methylprednisolone was reduced from 1.79 to 0.91 mg/kg/d within ECP treatment period (p=0.000012), (Figure 1). IST was discontinued in 24% of cases and reduced in 38%. Overall response rate was 62% with complete response (CR) in 7 patients (33%) and partial response (PR) in 6 (29%). CR in skin, liver and gut was 50%, 16.7% and 25%, respectively. PR in skin, liver and gut was 37.5%, 0% and 12.5%, respectively. Among 7 patients after haploidentical HSCT 5 patients responded (2 CR and 3 PR). Eleven patients (52%) were alive at a median follow-up

Figure 1. The average methylprednisolone daily dose at the onset and at the discontinuation of extracorporeal photopheresis.

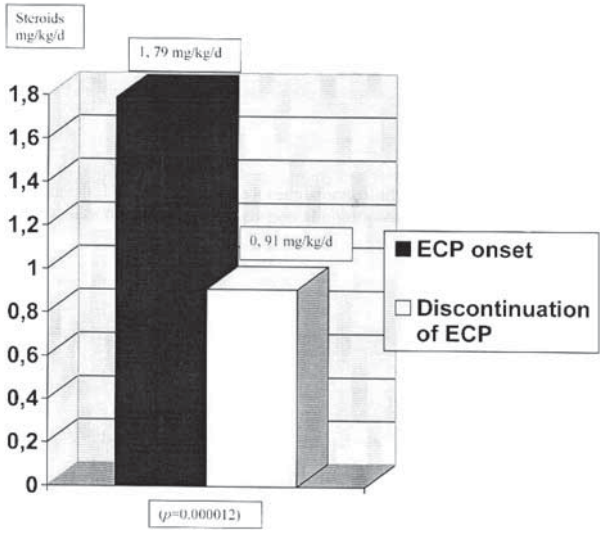
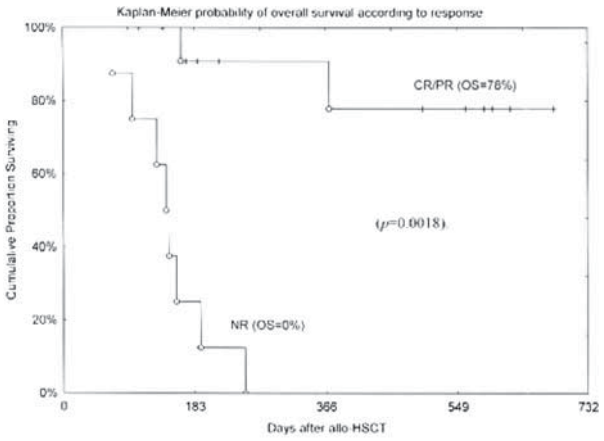


Figure 2. Kaplan-Meier probability of overall survival in patients with steroid-refractory acute GVHD after extracorporeal photopheresis.




CR-complete response, PR-partial response, NR-absence of response

of 382 days (range125-668) after allogeneic HSCT. In patients with CR or PR of steroid-refractory aGVHD Kaplan-Meier probability of overall survival at 1.5-years was 78% compared to 0% in patients without response (p=0.0018), (Figure 2).
Conclusion. ECP in steroid-refractory aGVHD may be used in combined IST with maximal effect in skin involvement. It demonstrated steroid-sparing effect in aGVHD patients. Our series also showed feasibility of ECP in aGVHD after haploidentical HSCT.

**P552**
**18F-FDG PET scanning in graft-versus-host disease after allogeneic stem cell transplantation**
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Background: Diagnosis and onset prediction of Graft-versus-host disease (GvHD) after allogeneic stem cell transplantation (AlloSCT) as well as therapy monitoring remains a challenging issue.
Aim: To evaluate the role of positron emission tomography (PET) using the positron-emitting radionuclide 18F labeled with Fluorodeoxyglucose (18F-FDG) for the assessment of GvHD after AlloSCT.
Methods: We retrospectively studied all patients with lymphoma or multiple myeloma who underwent AlloSCT between 2004 and 2009. We included all 36 patients in whom a PET scan before transplant was performed for staging purposes and who had at least three follow up PET scans after transplantation. Overall 8 non-Hodgkin lymphoma, 20 Hodgkin lymphoma, 8 multiple myeloma were reviewed. PET scans were blindly reviewed by two experienced nuclear medicine physicians. Scans performed at the time of GvHD were compared with scans obtained before transplant and during clinical remission of GvHD, as well as with scans performed in patients without GvHD. Pathological FDG uptake in GvHD target organs was recorded.
Results: Overall 25/36 patients developed GvHD (22 acute, 9 chronic) while 11 remained GvHD-free during the time of follow up. In 11/25 patients one or more PET scans were performed during the period of active clinical GvHD. In 7/11 cases pathological FDG uptake was detected in the gut (n=6), the liver (n=1) and the mouth (n=1). Six out of 11 patients had GvHD related diarrhea at the time of PET analysis. In these six cases there was intense FDG uptake in the bowel in comparison to the scans obtained in the same patients at GvHD-free periods. In 2 patients, PET analysis showed pathological FDG uptake days before the appearance of gastrointestinal symptoms. A strong decrease in bowel FDG uptake was observed in 5/6 patients responding to therapy. PET positivity was present in one of 3 cases with pathologically confirmed liver GvHD, and in one of 5 patients with oral GvHD. Among the 11 patients who did not develop GvHD, 2 patients showed a faint FDG uptake in the gut, that was also present in pre-transplant scans. Overall considering sites of documented GvHD, PET was positive in all patients with gut GvHD (n=6), in 1/3 with liver GvHD, in 1/5 with oral GvHD, and false positive in 2 patients without GvHD.
Conclusion: Our preliminary data show that together with clinical findings, FDG PET could be useful in diagnosing and monitoring GvHD, especially at bowel level.

**P553**
**Current practice in diagnosis and treatment of acute graft-versus-host disease – results from a survey among German-Austrian-Swiss transplant centres**
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Acute graft-versus-host disease (GVHD) is diagnosed clinically but histopathological confirmation is frequently warranted due to its unspecific nature of symptoms. We performed a survey on current practice for diagnosis and treatment of acute GVHD. The survey was returned by 31 (43%) of 72 centers contacted in Germany, Austria and Switzerland. Three paediatric centers answered as requested only the histologic part of the survey.
Results: In the presence of diarrhoea and decreased oral intake after engraftment only 3 centers (10%) do not perform endoscopy prior to immunosuppressive treatment, 13 centers perform colonoscopy, 8 colonoscopy and gastroscopy, 3 sigmoidoscopy and gastroscopy, 2 gastroscopy alone and 2 sigmoidoscopy alone. In the presence of a skin rash with the differential diagnosis of GVHD versus drug reaction 11 centers (35%) perform a skin biopsy upfront, 14 apply topical steroids with discontinuation of the suspected drug and perform a skin biopsy only if this strategy fails and 3 centers perform a skin biopsy only after failure of systemic steroids. Three centers do not perform a skin biopsy. In the presence of rapidly increasing cholestasis occurring without any other signs of GVHD 10 centers (32%) perform a liver biopsy upfront and 12 only after failure of steroid treatment while 9 centers do not perform a liver biopsy. Nineteen centers (61%) use a percutaneous, 11 a transvenous approach and one a mini-laparoscopy for liver biopsies.
First-line treatment of acute cutaneous GVHD stage 1 consists of topical steroids alone in the majority of responding centers (n=21, 68%), while isolated cutaneous GVHD stage 3 is treated with systemic steroids (prednisolone <0.5 mg/kg n=2, 0.5-1.0 mg/kg n=10, >1.0-2.5 mg/kg n=16) without or with topical agents (steroids n=10, calcineurin inhibitors n=3). In the presence of gastrointestinal manifestations of acute GVHD 8 centers (26%) add topical steroids to systemic ones and 3 consider topical steroids as the only treatment for mild gastrointestinal and cutaneous GVHD. The choice of agent for 2nd-line treatment as well as the sequence of administration are extremely heterogeneous. Most frequently applied are mycophenolate mofetil (n=11), etanercept (n=7), and extracorporeal photopheresis (n=6).

Conclusion: Histopathological confirmation of acute GVHD is part of clinical routine. While 1st-line treatment of acute GVHD is rather homogeneous, 2nd-line treatment is heterogeneous due to the lack of controlled data.

**P554**
**Kynurenine serum levels for monitoring acute graft-versus-host disease**
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Tryptophan, an amino acid essential for T-cell proliferation, is oxidized by indolamine 2,3-dioxygenase to kynurenine and its metabolites which also have immunomodulatory properties such as suppressing lymphocyte responses particularly by sensitizing them to apoptosis. It has been shown recently that tryptophan catabolism is associated with acute graft-versus-host disease (GvHD) by measuring the major tryptophan metabolites in urine and intestinal biopsies. We have addressed the question if tryptophan and its catabolites can be used to monitor acute GvHD when measured in serum.
Concentrations of kynurenine and tryptophan were analyzed by high-pressure liquid chromatography in sera of 64 patients prior to conditioning, on the day of stem cell transplantation (SCT) and at various time-points thereafter. Neopterin was determined by a radioimmunoassay. Statistical significance between patients developing GvHD and patients without this complication at the various collection times was estimated by Wilcoxon-signed-rank test.
In general, kynurenine levels were always higher in patients with acute GvHD grade II-IV (n=31) than in patients without GvHD (n=33). Patients with acute GvHD showed a first peak on the day of SCT, followed by a drop during the aplastic phase and again an increase until day 20. On that day, a mean concentration of 4,54±0,57 µmol/L kynurenine was detected in patients with acute GvHD in comparison to 2,72±0,25 µmol/L in patients without acute GvHD (p=0,007). These results were even more pronounced in patients experiencing acute GvHD grade III-IV (n=19). Accordingly, neopterin levels were also steadily higher in patients with acute GvHD than in patients without this complication. The highest levels were detected on day 60 in patients with acute GvHD grade III-IV (62,55±9,1 nmol/L versus 22,27±3,89 nmol/L in patients without GvHD; p=0,001). In contrast, tryptophan levels did not significantly differ between patients with and without acute GvHD. This may be explained by the tryptophan-containing parenteral nutrition applied in the majority of patients irrespective if they experienced acute GvHD or not.
In summary, significantly higher levels of the tryptophan metabolite kynurenine were detected in patients with acute GvHD so that kynurenine may be considered as a further biomarker for the detection of this complication.

**P555**
**Phenotypic analysis and single-cell gene profiling of human regulatory T-cell subsets in human graft-versus-host disease**
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Acute graft versus host disease (aGVHD) is an important cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (alloHSCT). CD4+FOXP3+ regulatory T cells (Treg), a population with potent immunosuppressive properties, may offer a new way to prevent or treat aGVHD. Treg therapy has demonstrated high efficacy in mouse models; however, translation to human has been hampered by the identification of different subsets of human Treg. Based on the expression of CD45RA and HLA-DR, we identified 3 different subsets of human FOXP3+ Treg in healthy subjects' peripheral blood and in cord blood. All 3 subsets were suppressive *in vitro*. Gene expression profiling combined with global pathway analysis revealed clearly distinct immune signatures for each subset, which were validated by analysis at the single-cell level. Single-cell gene profiling also uncovered a striking heterogeneity of gene expression within these Treg subpopulations and revealed that cytokine-expressing Treg did not downregulate FOXP3 and other Treg markers.
We prospectively studied Treg subsets in 18 consecutive alloHSCT recipients' peripheral blood. Analysis was performed before steroid initiation in patients with aGVHD (n=7) and at hematopoietic recovery in the control group (n=11). First sample was analyzed a median of 20 days after alloHSCT (range: 11 to 36) with no difference between the 2 groups. Percentages of FOXP3+ cells in CD4+ cells were not significantly different in aGVHD patients and in the control group (10.4 and 12.6%, p=0.53). However, we observed in the aGVHD group a strong alteration of Treg subsets compared to the control group, with a pronounced bias towards an activated phenotype. RA-DR+ cells were significantly more represented among FOXP3+ T cells in aGVHD patients than in the control group (80.8 versus 53%, p=0.003). Conversely, RA-DR- and RA+DR- cells were more frequent in the control group than in patients with aGVHD (26.8 versus 10.6% and 13.5 versus 2.1%, p=0.014 and p=0.0012, respectively).
Our data suggest that frequencies of specific Treg subpopulations, rather than the frequency of the total pool of CD4+FOXP3+ Treg, is altered in aGVHD and may serve as a biomarker for this condition.

**P556**
**TH17 cells but not IFNgamma producing lymphocytes proportions are low in blood in aGvHD but both well correlate with FoxP3+CD4+ cells**
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CD4+ lymphocytes under the influence of local environment differentiate into populations able to generate IFNgamma (Th1), IL-17 (Th17) and those exerting regulatory role FoxP3+CD4+. All these populations were described as main players in aGvHD. Therefore, the understanding of the interplay between the main subsets of CD4 positive cells may better understanding pathomechanism of aGvHD.
We studied 80 pts (median age: 43 yrs), 13 pts manifested aGvHD at the time of hematological recovery and in 15 pts aGvHD was clinically apparent from 17 to 93 days post transplantation (median 31). All patients were bled at the first day of hematological recovery and then in one week intervals as well as on the first day of clinical manifestation of aGvHD. PBMC were stimulated with brefeldin-A, Ionomycin and PMA and then with BD Golgi Stop The cells were labeled for CD4 positivity (BD, CA) and the intracellular presence of IL-17A, FoxP3 (e-biosciences, CA) and IFNgamma (BD).
It was found that:
(i) Percentages of Th17 lymphocytes (CD4+IL-17+) were significantly lower at the day of aGvHD manifestation as compared to those lacking aGvHD at the similar time post transplant (0.059%±0.01 *vs* 0.094%±0.009, p=0.007, M-W U-Test). It was also seen when percentage contribution of IL-17+ cells to CD4+ lymphocytes were analyzed (median values: 0.44% *vs* 0.21% *vs* 0.19% for lacking aGvHD, having cutaneous and gut aGvHD, respectively).
(ii) Percentages of IFNgamma producing lymphocytes in blood were also lower in having then in lacking aGvHD patients

(median: 0.05% *vs* 0.09%, p=0.147). However, the difference was less pronounced and statistically not significant.
(iii) Notably, contribution of IL-17+ cells (r=0.811, p=0.015) and those IFNgamma+ cells (r=0.819, p=0.013) to CD4+ lymphocyte population were well correlated with proportions of FoxP3+ cells in CD4+ lymphocytes. This was seen in aGvHD cases but not in those without aGvHD.
It appears that Th17 lymphocytes but not those producing IFNgamma marginalize at the tissue site at the beginning of aGvHD. However, these two populations levels close correlation with proportions of FoxP3+CD4+ cells in aGvHD cases depicts that all of them play a role in alloreactivity post HSCT.
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**P557**

**IFN-gamma microsatellite polymorphism as a risk factor of post alloHSCT complications revisited – Polish Donor-Recipient Matching Study Group**

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Several publications including our studies claimed the presence of an association between IFN-gamma genotype and susceptibility to complications post alloHSCT. However, these studies lack clinical implications and are not included in current clinical recommendations. In order to provide additional information supporting or declining the significance of genotyping of IFN-gamma and other implicated cytokines a multicenter study, well standardized as to the clinical observation and involving only one laboratory for genetical work, was conducted in Poland. Here we present the results of a study on the influence of the microsatellite polymorphism (CA)n within the first Intron of IFN-GAMMA gene on the outcome of HSCT from unrelated donors. Three hundred and sixty patient-donor pairs were investigated recording the level of HLA matching in 5 loci (A, B, C, DRB1 and DQB1) and clinical outcome of transplantation. IFN-gamma was genotyped with the use of PCR-STR technique.
It was found:

1. The susceptibility to toxic complications appearing early post-transplant was associated with recipient but the risk of aGvHD with donor genotypes.
2. Severe toxic complications were more frequent in recipients being 2/2 homozygotes as compared to other genotype combinations (13/45 *vs* 25/181, p=0.016).
3. The presence of II-IV grade aGvHD was more frequent in patients if they received transplant from donors being 3/3 homozygotes (26/66 *vs* 65/259, p=0.021). The latter association was much stronger in recipients receiving 10/10 matched graft (17/46 *vs* 37/176, p=0.025) than in those transplanted from not so well matched donors (9/19 *vs* 26/80, p=0.223).
4. The presence of IgG antibody against CMV was more frequent in patients having as compared to those lacking allele 3 (179/229 *vs* 49/79, p=0.005).
5. Multivariate analysis supports the argument that IFN-gamma genotype plays an independent role as a risk factor of aGvHD (OR=2.23, p=0.036) but with a lower power than level of HLA matching (OR=0.47, p=0.022) and toxicity post-transplant (OR=2.78, p=0.005).

In conclusion: Toxic complications are influenced by factors associated with recipient and aGvHD with donor IFN-gamma genotypes. The genetic trait of IFN-gamma genotype as a risk factor of aGvHD is rather of a moderate power as is seen only in patients receiving 10/10 matched graft at the allele level.
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**P558**

**CTLA-4 gene polymorphisms influence susceptibility to acute graft-versus-host disease after allogeneic haematopoietic stem cell transplantation**

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Donor T lymphocytes play the crucial role in alloimmune recognition and their ability to detect non-self antigens can differentiate predisposition to acute graft versus host disease (aGvHD). The effective recognition and activation of naïve T-cells requires two independent signals. The first is an antigen-specific and sent via the TCR on T-cells. The second signal is critical for allowing full activation. Cytotoxic T-cell antigen (CTLA-4) is a co-inhibitory molecule which down regulates the T-cell activation. Polymorphisms in the CTLA-4 gene result in abnormal expression function as well as dysregulated trafficking of that molecule within cellular compartments. Moreover the human CTLA-4 gene is known as susceptibility region for autoimmune diseases.
Therefore we postulate that polymorphism in CTLA-4 gene in donors after HSCT might be associated with predisposition to aGvHD. Our attention has focused on four functional polymorphic sites: CTLA-4c.49A>G, CTLA-4g.319C>T, CT60, and Jo31 which have been reported as associated with altered immune responses.
Altogether 175 patients (74 female/101 male), 67 transplanted from related donors (RD-HSCT) and 108 unrelated donors (URD-HSCT) in Department of Hematology, and Bone Marrow Transplantation, Medical University of Silesia, Katowice, Poland in 2006-2009 yrs. were included in this study. Median age of patients was 33 (range 18-57). In 86 patients there were no aGvH symptoms, while in 87 patients aGvHD incidences have been observed.
The SNPs were genotypes with using allelic discrimination methods with the following TaqMan SNP Genotyping Assays: C__2415786_10 for c.49A>G, C__27834180_10 for g.319C>T, C__3296043_10 for CT60, C__30981406_10 for Jo31.
The increased frequency of CT60 [G] allele among patients with aGvHD I-IV was observed in whole group of patients (pts.) and in pts. after RD-HSCT, while it was not present in URD-HSCT pts. Similar trend was noticed for Jo31 SNP. The associations between CTLA-4c.49A>G[GG] genotype and aGvHD were presented in all HSCT patients and in pts. after URD-HSCT. The donor haplotype CTLA-4c.49A>G[A], CTLA-4g.319C>T[C], CT60[A], Jo31[T] was protective against aGvHD grade I-IV in whole studied group of patients (OR=0.60, p=0.025) and in group of pts. transplanted from related donors (OR=0.39, p=0.02).
Our study indicated that CTLA-4 gene polymorphism might be associated with occurrence of aGvHD, especially in recipients transplanted from HLA-identical sibling donors.

**P559**

**Effect of CCL5 polymorphisms on the risk of graft-versus-host disease in HLA-matched sibling allogeneic haematopoietic stem cell transplantation**

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Objectives: There have been few studies about the predictive marker of graft-versus-host (GVHD) disease in the patients treated with allogeneic hematopoietic stem cell transplantation (all-HSCT). The serum level of CCL5 (Chemokine [C-C motif] ligand 5), one of chemokine has been known to be correlated to the risk of GVHD. We investigated the association between CCL5 polymorphisms and clinical outcomes of allogeneic hematopoietic stem cell transplantation.
Methods: A total of 147 patients who underwent human leukocyte antigen (HLA)-matched sibling allo-HSCT were included in this study. Three CCL5 gene polymorphisms of -403G/A (rs2107538), -28C/G (rs2280788), and In1.1T/C (rs2280789) were genotyped, and the clinical outcomes such as GVHD and survival were analyzed.
Results: Acute GVHD of the intestine did not occur in the patients with a genotype of -403AA, -28GG, or In1.1CC (p=0.087, p=0.14, and p=0.17, respectively). After adjusted by the known GVHD-associated factors such as age, gender, stem cell source, transplantation method, and the stratified risk category of the disease, A GCT haplotype in a recessive model showed a significant harmful effect on the occurrence of acute GVHD of skin and liver (Hazard ratio [HR] = 3.49 and 3.22, 95% CI: 1.20–10.16 and 1.11–9.33, p=0.022 and 0.031, respectively). An AGC haplotype in a dominant model had a protective effect on the acute GVHD of the intestine (HR=0.19, 95% CI: 0.039–0.92, p=0.039). However, we could not found significant difference between CCL5 polymorphisms and clinical outcomes, such as the risk of chronic GVHD, hepatic veno-occlusive disesase (VOD), post-transplant infection, and treatment related mortality. Furthermore, the CCL5 polymorphisms of the donor were not significantly associated with the risk of any subtype of acute GVHD.
Conclusion: This study suggests that CCL5 polymorphisms of the recipient of allo-HSCT, rather than the donor, might be preferentially associated with the occurrence of acute GVHD of intestine and skin. Our result supports the hypothesis that CCL5 expression in the cells of the specific tissue, including tissue antigen presenting cells of the recipient rather than that in T-cell of the donor, plays an important role in the development of the acute GVHD.

**P560**

**Effect of the synthetic inhibitory oligonucleotide 2088 on intestinal graft-versus-host disease in mice**

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Introduction: Bacterial and viral substances exacerbate Graft-versus-Host-Disease (GvHD) after allogeneic stem cell transplantation. Our previous results showed an important role of CpG-DNA via toll-like receptor 9 (TLR9) signaling in the triggering and exaggeration of GvHD in mice and the efficacy of the synthetic oligonucleotide 2088 (iODN2088) as an inhibitor of TLR9 with *in vivo* effectiveness against intestinal GvHD.
Methods: Prior to transplantation (day 0), C57BL/6-wildtype (wt) mice received Treosulfan 2000 mg/kg on day -3 to -1 and Cyclophosphamide 200 mg/kg on day-1. Mice were transplanted with 5x10E6 bone marrow cells and 3x10E6 splenocytes of Balb/c-origin. Mice were applicated 200 µ g iODN2088 on day -3, 0, +3 as prophylactic regimen. Mice were sacrificed on day +9. Cytokine-capture assays, functional assays of splenocytes and histological staining were performed.
Results: Administration of iODN2088 had no influence on markers of T-cell activation such as interferon gamma, interleukin-2, or *ex vivo*-CFSE-proliferation. However, nitrite oxygen (NO) and infiltration of macrophages in the colonic epithelium was significantly lower. Furthermore, in the liver stainings, we observed an increase of heme oxygenase 1 (HO-1), which is considered to have immunosuppressive properties.
Conclusion: Administration of iODN2088 alleviates intestinal GvHD and results in improvement of inflammatory markers. However, it has no obvious effect on T-cells. The T-cell independent effect of this substance leads potentially to a synergy of this substance with T-cell inhibitors. This synergy is to be further investigated.

**P561**

**Combination of gut GvHD and CMV enterocolitis in allogeneic haematopoietic stem cell transplant patients: 10-year experience at a single centre**

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Background: Post-transplant diarrhea is one of a common complications in allogeneic hematopoietic stem cell transplantation patients and the differential diagnosis includes conditioning-related, graft versus host disease (GVHD), and infection including bacterial, fungal, and CMV colitis. We presented our experience of post-allotransplant gut GVHD and the chance of combination of gut GVHD and CMV enterocolitis.
Methods: We analyzed our patients suffered from gut GVHD and CMV enterocolitis after the treatment of allogeneic hematolpoietic stem cell transplantation. The diagnostic tools of these patients include clinical assessment, blood CMV PCR, and H.E. stain and CMV immunostain of specimens from panendoscopic or colonoscopic biopsy.
Results: Between March 2001 and November 2011, we treated 181 patients with hematoloietic stem cell transplantation with 108 allotransplant and 73 autotransplant. Over 95% of our patients and their healthy donors are positive for CMV IgG. After allotransplantation, we have 42 patients to have grade II~IV acute GVHD (38.9%) including 19 grade III~IV (17.6%) and 29 patients to have extensive chronic GVHD (26.8%). In these patients, there were 11 to have severe gut GVHD (10.2%) and 6 patients diagnosed to have CMV colitis (5.6%) and there were 4 patients to have both gut GVHD and CMV enterocolitis (3.7%).
Conclusions: For gut GVHD, it is usually diagnosed clinically and there is no definitive serum marker to make gut GVHD diagnosis. On the contrary to other CMV infection or reactivation, it is usually false negative for CMV PCR and needs stringent colonoscopic biopsy for diagnosis under H.E. stain and CMV immunostain in CMV enterocolitis. Although the incidence is not high, there were over one third of severe gut GVHD patients to have associated CMV colitis. It is hazardous to overlook the possibiltiy of associated CMV enterocolitis in gut GVHD patients when treated with intensive immunosuppressant alone. It is crucial to perform colonoscopic biopsy when a suspicious gut GVHD patients were seen.

**P562**

**Severe refractory Enteritis obliterans as a result of the IV grade acute graft-versus-host disease**

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Refractory Graft-Verus-Host disease (GvHD) is one of the significant complications of the allogenic hematopoietic stem

cell transplantation (HSCT). GI-tract GvHD is rather rare, but very severe form of this reaction. In case of Cl. difficile infection persistence the grade of GvHD increases.
Objectives: To present the two cases of the enteritis obliterans as a result of severe GvHD with the following infection conjunction.
Patients: Pt. 1–1 y.o. boy with WAS after matched unrelated HSCT. Conditioning: Treo 36 gr/sq.m., Flu 150 mg/sq.m. and ATG 90 mg/kg. GvHD prevention - tacrolimus, MMF. The dynamic high intestinal obstruction with hemorrhagic bowel syndrome and paresis was diagnosed on day +90. GvHD grade IV (liver, intestine and skin) therapy: corticosteroids, etanercept, cyclophosphamide, campath, rituximab, temsirolimus. The upper intestinal obstruction and severe enteritis obliterans with the duodenojejunal impassability was diagnosed on day +210. Death - on day +540, reasons: multiple organ failure, metabolic disturbances, chronic extensive GvHD, loss of transplant function. Autopsy showed the total enteritis obliterans (small and large intestines).
Pt. 2-3 y.o. girl with severe idiopathic aplastic anemia after the matched relative HSCT. Conditioning: ATG 100 mg/kg, Cph 100 mg/kg, Flu 100 mg/sq.m. GvHD prevention – cyclosporine, MMF. Hemorrhagic colitis was registered on day +19, refractory persistence of Cl. difficile - on day +39. Therapy: metronidazole i.v., vancomycin and rifamixin p.o. continuously - without positive dynamic. The upper intestinal impassability with the expressed thickness of mucosa, contact bleeding, obstruction in the distal departments of duodenum from day +70. Reasons of death (day +200): multiple organ failure, metabolic disturbances, chronic extensive GvHD.
Discussion: Despite of the realized adequate immunosuppressive and anti-infectious treatment the refractory complications developed. This condition is the incurable situation for all treatment options. The enteritis obliterans complicated by the infection is the most severe condition in the context of the intestinal GvHD. The area of the Treitz ligament is the most common place of lesions localization.
Conclusion: The cases show the importance of the adequate immunosuppressive and anti-infectious therapy in very severe intestinal GvHD. The intestine impassability is the most severe complication of such a type GvHD. The infection complicates the severity of this condition.

**P563**
**Effect of HLA allels on graft-versus-host disease occurrence in Turkish patients receiving a human leukocyte antigen-identical haematopoietic stem cell transplant**
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Genetic variations between individuals could induce immune responses and cause graft rejection or graft versus host disease (GVHD) after hematopoetic stem cell transplantation (HSCT). Accordingly, certains HLA alleles can be protective against GVHD whereas some provides succeptibility. HLAs are greatly polymorphic which vary also among different ethnic groups. The aim of this study is to examine the effect of HLA alleles that may be associated with high risk for development of GVHD, or protective against GVHD in Turkish patients underwent HSCT.
Patients and Methods: Medical records of patients who underwent allogeneic HSCT, between March 1993 and December 2011 were evaluated retrospectively in this study. A total of 216 patients and their respective donors of HSC were enrolled. 73 patients had either grades 0-I or grades II-IV acute GVHD and 60 patients developed chronic GVHD.The commonest HLA-A alleles were HLA-A2, A3, A9, and A24. The commonest HLA-B alleles were HLA-B5, B7, B12, B35, and B51. The most common HLA-DR alleles were HLA-DR2, DR5, DR6, DR7 and DR11.
Results: Patients with HLA-A24, HLA-A9 and HLA-B63 alleles had suffered from acute GVHD more frequently (P:0.020 and P: 0.027 P: 0.048 respectively). Low incidence of aGVHD in patients having HLA-DR17 allele suggested that this allele might be protective against development aGVHD (P:0.031). HLA-A24 increased the risk of development of cGVHD, while HLA-B44 allele was associated with lower incidence (P=0,048 and P=0,019).
Conclusion: Various analyses including distribution of HLA genes regarding balance of inhibitory and activating role in certain diseases showed some significant association. Our findings also suggest that some HLA alles has some impact on limiting or developing of GVHD.Thus, the heterogeneity in both the expression of these alleles and the association of these alleles with GVHD occurence could be relevant within a country such as Turkey. Identification of distinct association of GVHD with some HLA allels may allow us to stratify patients into categories that differ with respect to disease course, clinical pattern, and treatment response.

**P564**
**Different gene variants are associated with outcome of patients transplanted from HLA-identical unrelated donors compared to patients with sibling donors**
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There are many conflicting reports existing in which different gene variants have been proposed to have influence on outcome of transplant. Here, we evaluated in 314 pts and their HLA ident.URD after T cell repleted myeloabl.transplant and use of GVHD-prophyl.with MTX/CSA or CSA/MMF, 48 different gene variants from which were reported earlier to have influence on outcome of transplant. Thereafter, we compared the results with those obtained from 285 pts transplanted from HLA-ident. sib.donor with the same setting in a 2nd cohort.
aGVHD grade 2-4 was influenced adversely by gene variants on recipient-side of LTA (39.5% *vs* 28.3%, P=0.013), MBL2 codon550 (46.7% *vs* 30.6, P=0.029), MCP1 1543 C/T (68.6% *vs* 41.6%, p=0.036) and NFKBIL1 (50.8% *vs* 34.1%, P=0.018). The occurrence of sev.aGVHD grade 3-4 was influenced adversely by gene variants of MBL codon 550 (9.8% *vs* 22.8%, P= 0.025), MBL2 codon 4 (9.8% *vs* 36.3% P=0.04), LCT13910 (9.3% *vs* 25.7%, P=0.04) and CYP1B1 (8.4% *vs* 19.4%; P=0.05). Favorable effect was induced by IL6 on aGVHD 3-4 (3.7% *vs* 18.9%, P=0.039). Remarkable, none of these gene variants had an influence on aGVHD grade 2-4 or 3-4 in the SIB cohort.
Further, we found that the 5-year NRM rate was associated adversely with the detection of variants of IL16 (60.2% *vs* 34.1%, P=0.011) and MCP1 (58% *vs* 27.2%, P=0.025), which also influenced the 5-year estimate for OS (MCP1 1543 C/T 39.5% *vs* 52.8%, P=0.014 and IL16 46.1% *vs* 28.1%, P=0.03). On the donor side the occurrence of aGVHD grade 2-4 was influenced by MBL2 codon4 (69.2% *vs* 32.2%, P= 0.007), TLR2 (65.7% *vs* 40.9%, P=0.02), TLR5 (75% *vs* 42.2%, P=0.041). AGVHD grade 3-4 was influenced by IL23R favorably (0% *vs* 19.8%, p=0.01) and adversely by IL18 (10% *vs* 35.5%; p= 0.01).
The 5-year NRM was found to be associated with the detection of gene variants at the donor side of CCR5 2086 (53.2% *vs* 26.5%, p=0.01), CTLA4 (23.1% *vs* 44.2%, P=0.018), CYP1B1 (13.9% *vs* 26.3%, P=0.045), TLR 2 (33.9% *vs* 66.3%, P=0.025). Also, IL10 gene variants at donor side influenced the 5-year OS (23.25 *vs* 53.9%, p=0.035) as well as TLR2 (28.1% *vs* 49.7%, P0 0.04), IL18 Rap (40.3% *vs* 71.5%, P=0.036) and FAS (60.3% *vs* 35.6%, P=0.041).
Interestingly, many gene variants which had influence on the outcome of transplant in the SIB group as NOD2 or GSTP showed no effect on the outcome of transplant in the URD cohort.

Only gene IL10, IL23R and LCT 13910 remained to be important in this analysis.
In conclusion we report here that gene variants have only a moderate influence on transplant outcome.

**P565**
**Proteomic patterns in saliva of patients submitted to allogeneic stem cell transplantation for haematological malignancies**
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Graft versus Host Disease (GvHD) is one of the major life threatening complication after allogeneic stem cell transplantation (SCT), which develops in 30-80% of all SCT despite immunosuppressive prophylaxis.
Diagnosis of GVHD is mainly based on clinical features. Recently the application of proteomic tools, allowing screening for differentially expressed or excreted proteins in body fluids, could allow detecting specific biomarkers and whole saliva is highly attractive for the non-invasive specimen collection. In this aim we collected salivary specimens of 40 patients (pts) submitted to alloHSCT between December 2008 to March 2011 in our Institution. They were: 17M/23F, median age 46 years (range 16-65). Underlying diseases were: 20 AML, 6 ALL, 3 MM, 1 LLC, 5 LNH, 2 LH, 2 MDS and 1 IMF. Graft was obtained from sibling donor and MUD in 28 and 12 pts respectively. GvHD prophylaxis was performed with CSA-MTX in 20 pts, CSA-MMF in 14 pts; CSA and Campath in 3 pts, CSA in 1 pt and 2 pts received no GVHD prophylaxis.
Samples of whole saliva were mixed immediately in a 1:1 (v/v) ratio with aqueous 0.2% trifluoroacetic acid solution and centrifuged at 8,000 g at 4°C for 5 min. The obtained solutions were analyzed by High-Performance Liquid Chromatography (HPLC) coupled to ElectroSpray-Ionization Mass Spectrometry (ESI-MS).
Twenty-three out of 40 pts (57%) developed aGVHD, involving oral mucosa. The chromatograms of the acidic soluble fraction of salivary proteins of pts with aGVHD were compared to the asymptomatic ones. Different expressions of S100A8 (calgranulin A, M average 10834 Da; elution time 37.0 min) and S100A7 (psoriasin; E27->D variant, M average 11368 Da; elution time 40.4 min) were observed: 14 pts out of 23 with GVHD showed the presence of S100A8, while the protein was detectable in two pts without GVHD (p 0.001). S100A7 was absent in pts without GVHD and detectable in 11 pts out 23 with GVHD (p 0.0001). S100A8, together with S100A9 (calgranulin B), is part of an etero-dimeric leukocyte-derived protein called calprotectin, which is over-expressed in oral mucosal inflammation. S100A7 is highly expressed in keratinocytes derived from psoriatic skin and it is chemotactic for CD4+ T cells.
We found a statistically significant association between the presence of these proteins and the development of aGVHD, but further studies should clarify if these proteins could be considered either a marker of GVHD or an index of mucosal inflammation.

**P566**
**Serum proteomic profiling and the roles of Ceruloplasmin as biomarker for aGvHD following allogeneic haematopoietic stem cell transplantation**
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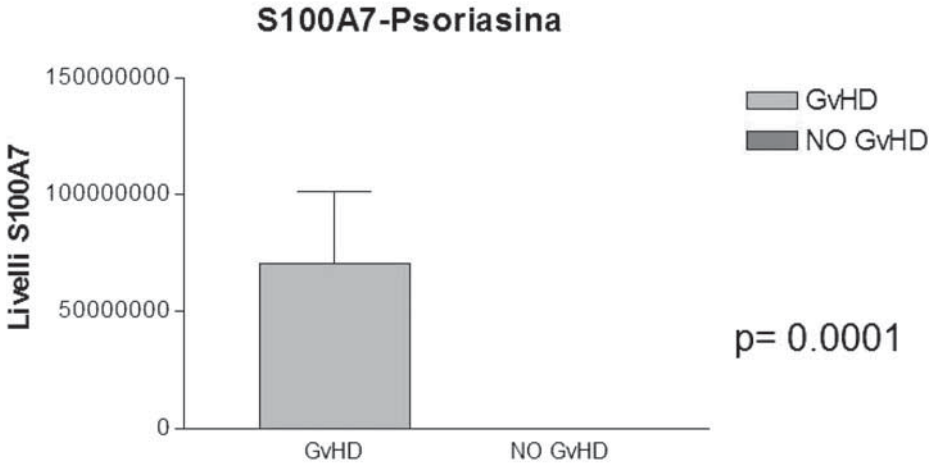
Objectives: Acute graft-versus-host-disease (aGvHD)is the major cause of non-relapse mortality of allogeneic hematopoietic stem cell transplantation (allo-HSCT). The diagnosis of aGvHD is based on clinical criteria and the biopsy of involved organs. Currently, few validated biomarkers are well established for the diagnosis of aGvHD. To seek for unknown proteins as serum biomarkers for aGvHD, the quantitative proteomics are used.
Methods: A quantitative proteomic approach, named isobaric tags for relative and absolute quantitation (iTRAQ) technology, was employed to screen differentiated expression proteins in peripheral blood of patients after allo-HSCT. We identified the differentiated expression proteins in the pathogenesis and recovery process of aGvHD and then validated one of the biomarker candidates, Ceruloplasmin, by ELISA in samples from 71 patients. 8 patients with HLA-matched donors received myeloablative regimens (modified Bu/Cy). 63 patients with HLA-mismatched/haploidentical donors received the modified Bu/Cy regimen and rabbit anti-human thymocyte immunoglobulin. Prophylaxis for aGvHD included cyclosporine A (CsA) and short-term methotrexate (MTX) with mycophenolate mofetil (MMF). The samples at multiple time points after conditioning of transplant, d-9, d-1, d7, d14, d21, d28, d56-60, d90-100, as well as at the onset and complete remission of aGvHD were measured.
Results: 21 kinds of proteins were up-regulated at the onset of aGvHD with proteomic profiling. Serum ceruloplasmin levels were significantly increased during the period of aGvHD (From median value 646.8 ug/ml to 1128.7 ug/ml, p<0.001), and decreased markedly as aGvHD resolved (decreased to 697.7 ug/ml, p<0.001). The serum ceruloplasmin levels at different time points after transplant in aGvHD(+) group were significantly

**[P565]**



[P566]

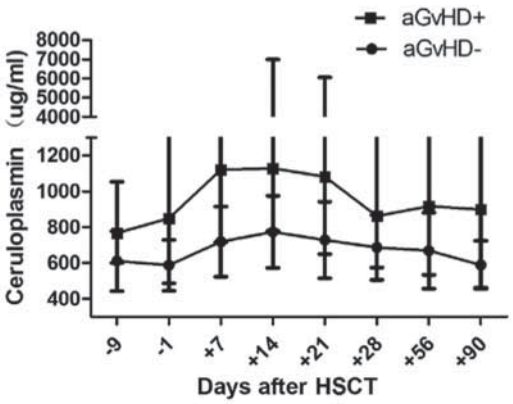



Figure 1. The kinetics of ceruloplasmin levels in patients with or without aGVHD after allo-HSCT. Compare with time point to time point of ceruloplasmin levels in aGVHD+ and aGVHD- groups (each group, n=20), d-1/d+7/d+14/d+21/d+56/d+90 P<0.05, ceruloplasmin level at different time point is shown by median value and standard deviation.

differentiated from those in aGvHD(-) group (p=0.0075), as shown in Figure 1. If ceruloplasmin levels elevated above 1000 ug/ml at d7, d14, d21 after HSCT, the subsequent probability of aGvHD would be remarkably increased.Serum ceruloplasmin levels were not correlated with grade of aGvHD, the target organ, as well as infection complications (p>0.05).
Conclusion: Our results suggest that serum ceruloplasmin was a serum biomarker candidate of aGvHD, ceruloplasmin levels more than 1000 ug/ml could be recognized as an "alarm" during the consecutive time points monitoring of it in patients after HSCT.

**P567**
**Close association between HHV6 reactivation and the occurrence of acute GvHD after allogeneic haematopoietic stem cell transplantation**

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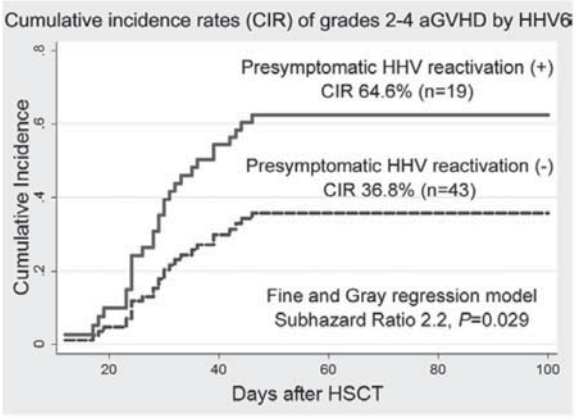
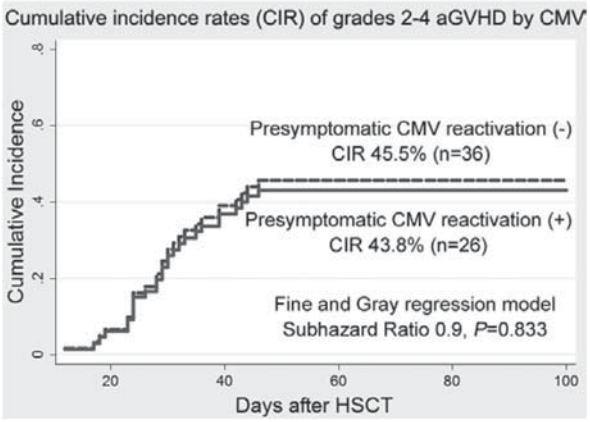
Objectives: To evaluate the association between viral reactivation and the occurrence of acute graft-versus-host disease (aGVHD) after allogeneic hematopoietic stem cell transplantation (HSCT).
Methods: Our study cohort consists of 62 consecutive adult patients who underwent HSCT at our institute between April 2008 and August 2011. Stem cell sources were bone marrow or peripheral blood, and cord blood transplantation was excluded. The conditioning regimen was myeloablative in 35 and reduced intensity in remaining patients. From a week before to 14 weeks after HSCT, peripheral blood samples were weekly drawn and real-time quantitative PCR assay for human herpes virus type 6 (HHV6) and cytomegalovirus (CMV) was done. Presymptomatic viral reactivation was defined as the detection of more than $10^2$ viral DNA copies/ml before the onset of grades 2-4 aGVHD as, in our experience, viral load more than $10^3$ viral DNA copies/ml is a signal for attracting attention to the development of HHV6- or CMV-associated diseases. The probabilities of aGVHD over time were estimated by cause-specific cumulative incidence rates (CIR). Fine and Gray regression model was used for the comparison of CIR between patient subsets.
Results: In our cohort, the incidence of grades 2-4 aGVHD was 45.2% (28/62) and that of grades 3-4 was 14.5% (9/62). Presymptomatic HHV6 and CMV reactivation was seen in 30.6% (19/62) and 41.9% (26/62), respectively. Patients with pre-



symptomatic HHV6 reactivation had a higher probability of subsequently developing grades 2-4 aGVHD than those without (CIR 64.6% *vs* 36.8%, subhazard ratio 2.2, P=0.029). Moreover, the incidence of grades 3-4 aGVHD was observed only among patients with presymptomatic HHV6 reactivation (CIR 45.3% *vs* 0%, P<0.001). In contrast, presymptomatic CMV reactivation did not associated with the occurrence of aGVHD. In a multivariate logistic regression model, HSCT from HLA-mismatched donor (odds ratio 5.1, P=0.021) and the use of myeloablative conditioning (odds ratio 5.1, P=0.021) were significant factors associated with presymptomatic HHV6 reactivation.
Conclusion: Presymptomatic HHV6 reactivation was associated with subsequent development of severe aGVHD. Routine monitoring of HHV6 load may be useful in the prediction of the onset of severe aGVHD.

**P568**
**Acute graft-versus-host disease: analysis of risk factors in 712 allo-HSCT**
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Objectives: Acute graft versus host disease (aGVHD) is the main complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Many risk factors have been reported in the literature: HLA disparity, age increase, sex disparity between donor and receptor, kind and status of the underlying disease, conditioning, use of radiotherapy, aGVHD prophylaxis, use of peripheral blood stem cells, performance status, donor/receptor CMV status, and race. We analyze the incidence and mortality of 8 of these risk factors in our serie of 712 allo-HSCT carried out in a single institution.
Patients and Methods: We analyze the influence of 8 variables (receptor age, sex disparity between donor/receptor, underlying disease, status of the underlying disease at transplantation, HLA disparity, conditioning regimen, source of hematopoietic stem cell and aGVHD prophylaxis) in our serie of 712 allo-HSCT (565 related and 147 unrelated) carried out in our Hospital between 1980 and 2010. The source of stem cells was bone marrow in 577 patients, peripheral blood in 97 and cord blood in 38. Statistical analysis were done with $\chi^2$ test, student t-test and Mann Withney test in univariate analysis and logistic regression test in multivariate analysis using the SPSS (17 version) statistical program.
Results: Both in univariate and multivariate analysis, we observe more incidence of aGVHD grade II-IV in age (>45 years: 57% *vs.* 39%; p=0,001), neoplasic *vs* non-neoplasic underlying disease (44 *vs.* 20%; p=0,001), donor non-HLA-identical sibling (54% *vs.* 38%; p<0,001) and the use of radiotherapy in the conditioning regimen (45% *vs.* 35%; p=0,019). In univariate analysis the use of ciclosporine plus MTX short course (OR 0,1; p=0,034) and HLA-identical sibling as donor (OR 0,2; p=0,02) were associated with lesser mortality due to aGVHD. Donor/receptor female/male, ciclosporine plus MTX short course and donor non-HLA-identical sibling were statistically associated with more incidence of aGVHD grade III-IV.
Conclusions: Our results show that receptor age >45y, neoplasic underlying disease, use of radiotherapy in the conditioning regimen and donor non-HLA-identical sibling are factors that predispose to more incidence of aGVHD. Nonetheless, only donor HLA-identical sibling, sex of donor/receptor different of the combination female/male and the use of ciclosporine plus MTX short course are associated statistically with lesser mortality due to aGVHD.

**P569**
**miR-146a expression in graft-versus-host disease**
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*Equal contributions

Background: Graft-versus-Host disease (GvHD) which is an inflammatory disease still remains a serious complication of allogeneic (allo-) haematopoietic stem cell transplantation (HSCT). MicroRNA (miR)-146a has been associated with disease activity in many inflammatory diseases and is involved in both innate and adaptive immune systems. Validated targets of miR-146a include IRAK1 and TRAF6, which are known to promote expression of interferon genes via the NF-kB pathway. MiR-146a has been shown to inhibit IRAK1 and TRAF6 as well as reduce the expression of specific cytokines (IL-2, -6 and -8) in aGvHD. The aims of this research were to investigate: (1) the relationship between miR-146a expression and the pathogenesis of GvHD, (2) the mRNA expression of known miR-146a targets; TRAF6 and IRAK1 in GvHD.
Methods: MiR-146a expression was assessed by qRT-PCR in skin biopsies obtained from *in vitro* skin explant assay (SEA) and HSCT patients. Skin biopsies in medium alone were used as the control for SEA samples. Similarly, skin biopsies from healthy volunteers were used as the control for clinical skin samples. MiR-146a expression was also analysed in whole

**[P569]**

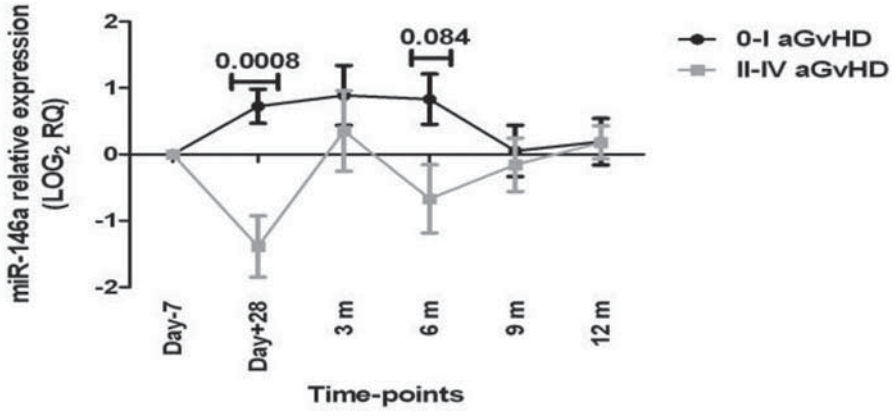


**Figure 1.** miR-146a expression in whole blood of 0-I aGvHD versus II-IV aGvHD patients.

[P569]

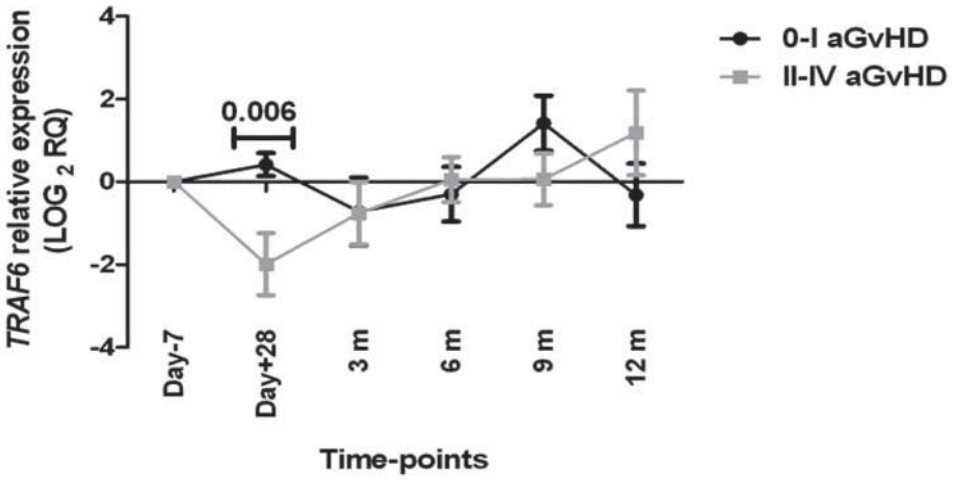


**Figure 2.** *TRAF6* expression in whole blood of 0-I aGVHD versus II-IV aGvHD patients.

blood taken from allo-HSCT patients (n=57) with and without GvHD and at set time-points pre- and post-transplant (Day-7 to 12 months). Target TRAF6 and IRAK1 mRNA levels were assessed in whole blood samples using Taqman gene expression qRT-PCR assays.
Results: There was no significant correlation between miR-146a expression and GvHR or GvHD severity in skin biopsies from SEA or HSCT patients. In whole blood, miR-146a (p=0.0008) and TRAF6 (p=0.006) expression was significantly down-regulated in II-IV aGvHD patients in comparison to 0-I aGvHD patients (Figure 1 and 2). However, no significant difference in miR-146a or TRAF6 expression was observed in whole blood with regards to chronic GvHD (cGvHD). IRAK1 expression did not significantly vary in either the aGvHD or the cGvHD cohort.
Conclusion: Low expression of miR-146a at 28 days post-transplant in whole blood obtained from II-IV aGvHD patients could be an early indicator of aGvHD severity. Results may suggest that miR-146a has a protective role as higher expression levels are observed in whole blood of 0-I aGvHD patients. Further investigations are required to examine protein levels of known miR-146a targets (TRAF6 and IRAK1), as well as the mRNA expression levels of additional potential miR-146a targets.

**P570**

**Acute graft-versus-host disease and expression of Toll-like receptors on peripheral blood cells after allogeneic stem cell transplantation**

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Introduction: Pattern recognition receptors such as Toll-like receptors (TLRs) play a key role in the cross-talk between innate and adaptive immune system. TLRs recognize pathogen-associated molecular patterns, such as common protein, carbohydrate or DNA/RNA pattern motifs. TLRs are also receptors for endogenous ligands and damaged tissue. Extracellular ligands are recognized by surface TLRs (TLR1, TLR2, TLR4, TLR5, and TLR6). Intracellular TLRs (TLR3, TLR7, TLR8 and TLR9) bind mainly to foreign nucleic acids and sometimes detect self DNA/RNA.
Aim of the study: Very little is known about expression and function of TLRs *in vivo* in patients who underwent allogeneic stem cell transplantation (SCT). The aim of this study was to evaluate the expression of TLRs on lymphocytes and monocytes in relation to the onset of acute graft-versus-host disease (GVHD).
Methods: The expression of TLRs on lymphocytes and monocytes was analysed by flow cytometry as mean fluorescence intensity at day +30 and at the onset of GVHD. Functional data were obtained by ELISA assay after TLRs activation. The cell supernatants were collected and assayed for TNF-alpha, IFN-gamma and MCP-1. Relative induction of these cytokines was calculated in relation with unstimulated controls.
Results: We analyzed 17 healthy donors and 34 patients. Acute GVHD developed in 19 patients (12 with grade ≥2). Clinical and transplant characteristics did not differ in patients with and without GVHD. Lymphocytes and monocytes of patients with acute GVHD showed higher levels of TLR5 (3,5±2,3 *vs.* 1,9±1,6 p=0,03; 25,8±25,9 *vs.* 9,0±5,0 p=0,02) and a decreased expression of TLR1 (2,5±2,8 *vs.* 4,3±2,8 p=0,02; 21,4±21,9 *vs.* 54,9±37,4 p=0,005) and TLR9 (63,8±30,4 *vs.* 111,1±62,9 p=0,03; 85,3±73,9 *vs.* 164,2±90,6 p=0,01). IFN-gamma relative induction post-stimulation of TLR2,3,4 and 9 was significantly decreased in patients with acute GVHD (p<0,04).
Conclusions: These results suggest that the innate immune response via TLRs activation could be involved in the development of GVHD. In particular, a decreased expression of TLR-9 (receptor of hypomethylated DNA) on lymphocytes and monocytes can promote TLR-7 activation, inducing type I interferons and other pro-inflammatory cytokines. TLR-1 and -5, which are ligands for bacterial cell wall, could also be involved in the pathogenesis of GVHD. Moreover, acute GVHD negatively correlates with IFN-gamma production upon TLR2,3,4 and 9 activation.

**P571**

**Positron emission tomography as a non-invasive modality to diagnose graft-versus-host disease of the gastro-intestinal tract: a paediatric experience**

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*The Children Hospital at Westmead (Westmead, AU)*

Introduction: Acute Graft versus Host Disease (GvHD) of the Gastrointestinal tract (GIT) tract can affect any section of the

gut and symptoms are nonspecific. Early recognition is important to start therapy promptly. The current standard for diagnosis is endoscopy of the upper and lower GIT, with histology of biopsies. The clinical and haematological status of the patient may make obtaining endoscopy and biopsy difficult and histological features are quite nonspecific. Involvement can be patchy and affected areas may be missed occasionally on endoscopy. As 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) detects mucosal inflammation, we describe its utility as a non-invasive test for gut GvHD and its correlation with histology.

Methods: Ten patients with clinical suspicion of acute gut GvHD between March 2009 to Oct 2011 underwent FDG-PET scanning and endoscopic biopsies of the upper and lower GI tract within 7 days of each other. All were evaluated for other causes for their symptoms before proceeding to PET scan and endoscopy. The final diagnosis of GvHD was made based on clinical symptoms, histology and response to subsequent therapy.

**[P571]**

**Table 1: Patient characteristics**

| S.No | Age (yrs) | Diagnosis | Conditioning | Type of Transplant | GVHD prophylaxis |
|---|---|---|---|---|---|
| 1 | 8 | AML in CR2 | Flu-Bu-Mel | Unrelated PBSC (9/10 match) | CD34 selection, Cyclosporine |
| 2 | 14 | ALL in CR2 | Flu-TBI-Cyclo | Unrelated Cord | Cyclosporine, MMF |
| 3 | 11 | ALL in CR2 | Flu-TBI-Thio-Cyclo | Unrelated PBSC (10/10 match) | CD34 selection, Cyclosporine |
| 4 | 10 | AML in CR1 | Bu-Mel | MSD (marrow) | Cyclosporine, MTX |
| 5 | 9 | Aspartylglucosaminuria | Bu-Cyclo | MSD (marrow) | Cyclosporine, MTX |
| 6 | 5 | ALL in CR1 | TBI-Thio-Cyclo | Sibling (7/8 match marrow) | Cyclosporine, MTX |
| 7 | 5 | Aspartylglucosaminuria | Bu-Cyclo | MSD (marrow) | Cyclosporine, MTX |
| 8 | 6 | AML in CR1 | Flu-Bu | MSD (marrow) | Cyclosporine |
| 9A* | 14 | CML in Blast Crisis | TBI-Thio-Cyclo | MSD (marrow) | Cyclosporine, MTX |
| 9B* | 14 | CML in Blast Crisis | TBI-Thio-Cyclo | MSD (marrow) | Cyclosporine, MTX |
| 10 | 13 | AML in CR2 | Flu-Bu | Unrelated Marrow (10/10 match) | Cyclosporine, MTX |

*Same patient

**Abbreviations:** AML: Acute Myeloid Leukaemia, ALL: Acute Lymphoblastic Leukaemia, CML: Chronic Myeloid Leukemia
CR: Complete Remission, Flu: Fludarabine, Bu: Busulphan, Thio: Thiotepa, Cyclo: Cyclophosphamide, TBI: Total body irradiation
PBSC: Peripheral Blood Stem Cells, MSD: Matched Sibling Donor, MMF: Mycophenolate Mofetil, MTX: Methotrexate

**Table 2: Results**

| S.No | Biopsy suggestive of GvHD | PET scan uptake | PET uptake grading | GVHD | Other sites of GVHD (day after transplant) | Treatment given | Response to Rx |
|---|---|---|---|---|---|---|---|
| 1 | Rectum and Sigmoid | Ascending colon and rectum, patchy in small bowel | 1 | Gut Stage 1, Overall GrII | Skin Stage 2 | Budesonide | CR |
| 2 | Colon and rectum | Whole colon, patchy in small bowel | 2 | Gut Stage 2, Overall Gr III | Nil | Systemic Steroids | CR |
| 3 | Duodenum, Descending colon, Sigmoid and Rectum | Whole of large bowel | 2 | Gut Stage 1, Overall GrII | Skin Stage 2 | Systemic Steroids | CR |
| 4 | Stomach and Colon | Whole of large bowel | 2 | Gut Stage 1, Overall GrII | Nil | Budesonide | CR |
| 5 | Stomach ; Colon and Duodenum : Mild changes | Rectum and sigmoid | 1 | Gut Stage 2, Overall Gr III | Skin Stage 2 | Systemic Steroids | CR |
| 6 | Duodenum and Rectum: (after three days of 1mg/kg steroids) | Rectum and sigmoid (after three days of 1mg/kg steroids) | 2 | Gut Stage 1, Overall GrII | Skin Stage 1 | Systemic Steroids and sirolimus | CR |
| 7 | Stomach | Descending colon, rectum , sigmoid | 2 | Gut Stage 1, Overall GrII | Nil | Systemic Steroids | CR |
| 8 | not suggestive of GVHD | No uptake | 0 | Nil | Nil | Nil | NA |
| 9A* | not suggestive of GVHD | No uptake | 0 | Nil | Nil | Nil | NA |
| 9B* | Stomach and duodenum | Uptake in stomach and large bowel and patchy in small bowel | 2 | Gut Stage 1, Overall GrII | Nil | Budesonide | CR |
| 10 | Stomach | Large bowel and rectum | 2 | Gut Stage 2, Overall Gr III | Skin Stage 1 | Systemic Steroids | CR |

*same patient
Abbreviations: GVHD: Graft vs Host Disease, CR: Complete Resolution
FDG uptake grading: 0- Normal; 1-Mildly Pathologic; 2-Severly Pathologic

Results: Ten patients (median age 10 years) had PET scan and endoscopic biopsies for suspected GvHD. One patient was evaluated twice. The clinical data is shown in Table 1 and 2. Out of the total of 11 evaluations, 9 were suggestive of GvHD on histology and PET: 9 showed increased uptake in the GIT suggestive of GvHD. Two patients negative on histology as well as PET were labelled as no gut GvHD and recovered with supportive treatment alone. One of these patients had later new symptoms and was then positive on histology and PET. There was 100% concordance between the histology of the biopsy specimens and the PET scan in diagnosing GvHD of GIT, although in 2 patients the biopsy and PET positivity were at different sections of the GI tract. All the patients with positive histology and PET scan showed complete resolution of the symptoms after GvHD treatment.
Conclusion: FDG-PET demonstrates significant potential as a non-invasive technique for evaluation of suspected acute GvHD of the GIT. Moreover PET can guide the endoscopists to target biopsies from the more affected areas. FDG-PET as a sole modality in addition to clinical symptoms for diagnosing acute gut GvHD needs further evaluation as the FDG uptake in patients with intestinal inflammation due to other causes (like CMV enteritis) may look same.

**P572**

**An association between skin homing T-cells and graft-versus-host disease**

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Objectives: Cutaneous lymphocyte antigen (CLA) or CCR10 bearing T cells have been implicated in the development of GVHD [1,2]. This study examined the kinetic changes of CLA+ and CCR10+ T cells and their association with GVHD after allogeneic haematopoietic stem cell transplantation.
Methods: Flow cytometry analysis was performed on 95 PBMC samples from 20 patients.
Results: The levels of CLA+CD4 T cells were significantly higher for patients with aGVHD than those without at 100 days post transplant followed by a decline to pre-transplant level at 6 & 12 months. A second peak was apparent in aGVHD patients at 9 months although not significant. Similar kinetic patterns were observed for CLA+CD8 T cells but to a lesser extent (Figure 1a). Patients with de novo chronic GVHD showed a sharp increase in CLA+CD4 T cells at day 28, which remained significantly high during the 12-month period. The levels of CLA+CD8 T cells remained comparable for patients with or without cGVHD at all time points (Figure 1b). There was a trend showing increased levels of CCR10+CD4 and CD8 T cells for patients with aGVHD at day 28 or 3 months as well as raised CCR10+CD4 T cells for patients with cGVHD at most time points, yet no statistical significance was attained. To assess CLA expression and T cell activation we compared the kinetic patterns of CLA+ and CD25+ T cells. There was a trend showing higher levels of CD25+CD4 and CD8 T cells in patients with aGVHD than those without. However the levels of CD25+CD4 and CD8 T cells never exceeded the pre-transplant level. In addition the levels of CD25+CD4 T cells remained high even after the resolution of aGVHD (Figure 2a). Patients with cGVHD had significantly higher levels of CD25+CD4 T cells compared to those without while the levels of CD25+CD8 T cells were comparable regardless of cGVHD status (Figure 2b). Interestingly a sharp decline of CD25+T cells was observed in all patients at day 28 except those with aGVHD, maybe indicating a lack of response to cyclosporine A.
Conclusions: Our data suggest that increased frequency of CLA+CD4 T cells in PBMC maybe an informative indicator for acute and chronic GVHD. CLA+T cells do not follow the same kinetic patterns as CD25+T cells post transplant, suggesting they may have diverse functional roles in GVHD. The association of CCR10+T cells and GVHD is inconclusive in this small cohort of patients.
[1] Tsuchiyama *et al.* 2009, Bone Marrow Transplantation
[2] Faaij *et al.* 2006, British Journal of Haematology.

**[P572]**

Figure 1. Kinetic changes of CLA+ T cells after allogeneic HSCT

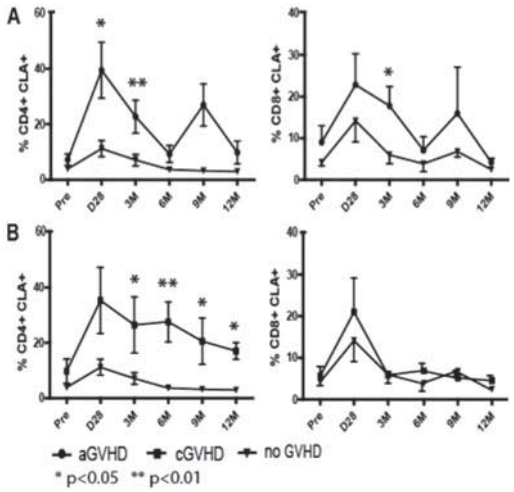
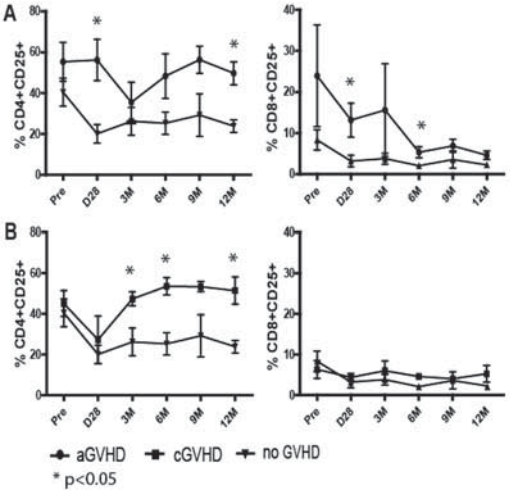



Figure 2. Kinetic changes of CD25+ T cells after allogeneic HSCT




**P573**

**An easy-to-use score to predict outcome of acute graft-versus-host disease**

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Purpose: Acute Graft versus Host Disease (aGvHD) is a major cause of mortality after allogeneic stem cell transplantation (SCT). Current promising attempts to predict outcome of aGvHD include gene-expression profiling. However, for rapid clinical decision-making additional, easy-to-use clinical parameters may be helpful. As thrombocytopenia and hypalbuminemia predict outcome in chronic and aGvHD, we analysed the combined influence of these factors on outcome.
Methods: We retrospectively analysed 42 patients with hematological malignancies who underwent SCT in our centre and were diagnosed with aGvHD. Albumin (Alb) and thrombocyte count (TC) at time of initiation of aGvHD treatment were categorised: Alb low (<28 g/l) *vs.* high (≥28 g/l) and TC low (<100 Gpt/l) *vs.* high (≥100 Gpt/l). For preliminary analysis overall survival (OS) served as the primary endpoint. Median follow up time was 24 months (interquartile range; IQR 20).

Results: Donors were matched related in 10 (24%), matched unrelated in 23 (55%) and mismatched unrelated in 9 (21%) patients. Immunosuppression comprised of cyclosporine (CsA) and mycophenolic acid in 31 (74%) and of CsA and methotrexate in 11 (26%) patients.
Acute GvHD grade I-II and grade III-IV occurred in 25 (64%) and 14 (36%) patients. Median onset of aGvHD was day +27 (IQR 21.5). 13 patients (13%) had self-limiting disease. In 19 patients (45%) treatment comprised prednisolone 2-3 mg/kg and in 8 increased CsA dose (19%). At onset of aGvHD, 18 (43%) and 23 (57%) patients had low Alb and TC, respectively. A significant association between low Alb (HR 4.8, 95%CI 1.9-12) and in trend low TC (HR 2.3, 95%CI 0,6-4.3) with shorter OS was seen. This association remained significant for Alb after stratification for aGvHD grade I-II (HR 5.7 95%CI 1.4-22) and III-IV (HR 10 95%CI 1.1-91). A score comprising Alb and TC distinguished three survival groups. In comparison to patients with high Alb and TC, patients with either low Alb or low TC (HR 2.5 95%CI 0.7-8.8) or with low levels of both markers 6 (1.9-20) had worse outcome. At the meeting, additional data will be presented from a larger cohort and with response to therapy as well as treatment-related mortality as endpoints.
Conclusions: This easy-to-use score could help in clinical stratification of patients with aGvHD. Validation of the score in larger patient cohorts is needed and may help to develop trials for risk dependent treatment strategies of aGvHD.

**P574**

**Plasmacytoid dendritic cells and Th17 immune response contribution in gut acute graft-versus-host disease**

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Previous studies established an important role of Th1 cells in aGVHD pathophysiology after allo-SCT. The role of proinflammatory Th17 cells in aGVHD has been demonstrated in mouse models. However, their contribution in human gastrointestinal (GI) aGVHD remains unclear. The same, the role of PDC (the professional type I IFN-secreting cells), which play an important role in triggering Th17-related cytokines is not established in aGVHD. This report investigated the role of Th17 cells and PDC in GI biopsies taken from patients with or without aGVHD.
The cohort included 21 patients who underwent allo-SCT for different hematological malignancies (n=19) and severe aplastic anemia (n=2), with a median age of 53 (range, 16-69) y. The stem cell source was PBSCs in 19 cases, CB in 2 cases and BM in 1 case. 10 patients received transplant from a MRD, and 11 patients from a MUD. A RIC regimen was used in the majority of cases (n=19). Immunohistochemistry was performed on deparaffinized tissues sections. A quantitative evaluation of antigens expression was performed by counting the number of positive cells in the biopsy.
In this cohort, based on standard pathology criteria, 16 patients had a histologically proven GI aGVHD, biopsies were taken before initiation of systemic corticosteroid therapy. The remaining 5 patients did not have histological or clinical signs of aGVHD and were used as controls. In order to identify the Th17 cell, biopsies were tested for expression of the CD161 and CCR6 markers, and RORgammaT, the key transcription factor for the differentiation of Th17 cells. Significantly higher numbers of RORgammaT+ and CD161+ cells were counted in the intestinal mucosa of patients with aGVHD (p=0.016 and p=0.009 for RORgammaT and CD161 expression respectively).
Given the role of PDCs in triggering Th17-related cytokines, we determine the proportion of PDCs in the GI biopsies. This analysis showed a significant increase of CD123+ PDCs in patients with aGVHD (p=0.017).
We show that Th17 cells and PDC infiltrate intestinal biopsies from patients with aGVHD, suggesting a potential new pathophysiological link between PDCs and Th17 response in the context of GI aGVHD. Although the exact mechanism that links type I IFN production to PDC-mediated Th17 responses is still unclear in aGVHD, these data raise the prospect of future innovative approaches to optimize immunosuppression regimens for the treatment of aGVHD by targeting PDCs and the Th17 response.

**P575**

**Automated enumeration and characterisation of circulating endothelial cells as a biomarker of endothelial damage in patients undergoing allogeneic stem cell transplantation: correlation with graft-versus-host disease onset**

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AlloBMT can be burdened by life-threatening complications, being GVHD the major cause of morbility and mortality. Clinical and physio-pathological evidences have shown that vascular endothelium could be a target of GVHD in very early phase; therefore markers of endothelial damage are warranted as valuable support in GVHD diagnosis. Several soluble factors have been tested as endothelial injury biomarkers, but any proved sufficient specificity and reliability to enter clinical practice. We conducted a study with primary endpoint to identify and count CEC in peripheral blood of patients undergoing alloBMT as a function of endothelial damage.
The CellSearch System® was used to capture and enumerate CEC. Magnetic particles conjugated to anti-CD-146 are used to capture CEC from 4.0 ml of blood. Enriched cells are stained with DAPI and anti-CD-105-PE antibody. APC conjugated anti-CD-45 is used to exclude leukocytes. Enriched and stained cells are dispensed into a MagNest® cartridge for magnetic mounting. The cartridge is scanned and individual images of cells are presented for review and scored as CEC, based on CD146+, CD105+, DAPI+ and CD45- phenotype and cell morphology. Patients undergoing alloBMT were tested before, after the conditioning regimen and at weekly intervals till day +100 from alloBMT. Ten healthy subjects have served as controls.
We enrolled 14 patients with hematologic neoplastic diseases (3 HD, 3 AML, 3 ALL, 3 MM, 1 CLL, 1 SAA) undergoing alloBMT from either HLA-matched familial (n=8) or unrelated donor (n=6). The study period is actually ranging from day +7 (n=5), to day +50 (n=2) and day +70 (n=1) post-transplant, with six patients having already concluded the evaluation at day +100. The median CEC/ml pre-alloBMT was 15 (n=14, range 4-94), going up to 30 (n=13, range 11-648, P=NS) CEC/ml at the end of the conditioning regimen; at time of engraftment the median CEC/ml was 74 (n=8, range 47-276, P=0.0019), while at GVHD onset was 56 (n=4, range 45-75, P=0.013). At GVHD response the median CEC/ml went down to 22 (n=4, range 6-86), being statistically significant (P<0.01) in comparison to CEC count at GVHD onset. The median count in 10 healthy subjects was 2 (range 1-14) CEC/ml.
We showed a statistical significant increase in CEC numbers at GVHD onset, with a normalization at treatment response. Our results need to be confirmed in prospective clinical trials to establish the prognostic and predictive value of CEC numbers on GVHD diagnosis.

**P576**

**Faecal calprotectin and alpha-1 antitrypsin predict severity and response to corticosteroids in gastro-intestinal graft-versus-host disease after allogeneic haematopoietic stem cell transplantation**

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Diagnosis of GI-GVHD is based on clinical symptoms and histological findings. Fecal CAL, AAT and elastase are noninvasive biomarkers used in the screening of intestinal inflammation, protein-losing enteropathy and exocrine pancreatic dysfunction, respectively. They have not been yet validated in the context of HSCT and we aim to evaluate them as diagnostic and prognostic biomarkers of GI-GVHD. From 09/08 to 12/10, 72 patients (pts) were evaluated: 51 with GI-GVHD, 9 with GI infection and 12 without GI symptoms. At first symptoms fecal samples were collected to measure CAL, AAT and elastase concentrations. Prognostic value of markers was evaluated by their association with complete response (CR) and steroid-refractory (SR) GVHD using a multiple logistic regression. Cumulative incidence (CI) functions were compared using Gray's test and adjustment was performed using the Fine and Gray method. CAL and ATT concentrations were higher in pts with GI symptoms than in controls. The concentration of elastase in pts with GI-GVHD was decreased but associated with high levels of CAL and alpha1-AT attesting from mucosal damage possibly not related to a pancreatic dysfunction. Pts with stage 1 GI-GVHD had similar concentrations of CAL and AAT than controls. Initial concentrations of CAL ≥100 µg/g and AAT > 1.5 mg/g decrease the probability of CR after treatment (56% *vs.* 86%, p=0.005; 67% *vs.* 90%, p=0.00007, respectively). Similarly, high level of CAL and AAT predict a 93% and a 72% probability of SR-GVHD (p<0.0001). CAL and AAT concentration were independently and exclusively associated with a lower probability of CR (HR: 0.47). The model selection procedure also identified a mode with both variables together with stage > 2 GI-GVHD as best predicting SR-GVHD, although marginal testing did not yield a significant odds ratio (OR) for AAT (CAL: OR 17.1 [p=0.016]; GI stage III-IV: OR 7.11 [p=0.043]; alpha1-AT: OR 3.67 [p=0.11]). Interestingly, excluding GVHD stage from the model, CAL and AAT concentration could also independently predict SR-GVHD (OR:15.8, p=0.015 & OR:4.93, p=0.038). Our results showed that fecal CAL and AAT levels are biomarkers of GI-GVHD, particularly in GI stage > I. Furthermore, an increase level of those markers predicts the response to treatment. Their use as predictors from severe GI-GVHD are very promising including in patients in whom GI-GVHD stage or histology are not available.

**P577**

**The impact of grade II acute GvHD on relapse and survival after cord blood transplantation**

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Introduction: Although incidence and severity of acute graft-versus-host disease (AGVHD) differ depending on donor stem cell source, impact of AGVHD upon disease relapse and survival has not been analyzed enough in cord blood transplantation (CBT) with single graft setting. In this study, we have investigated the effects of AGVHD on outcomes of CBT.

Patients and Methods: We have analyzed 135 consecutive patients with acute leukemia (AML N=94, ALL N=41) who underwent CBT after myeloablative conditioning containing 12 Gy total body irradiation in the Institute of Medical Science, University of Tokyo between August 1998 and January 2011. Median age was 39 (range, 16-55) years. Seventy-nine patients were in first (CR1) or second (CR2) complete remission and 59 were in other disease status. All patients received a single cord blood unit and AGVHD prophylaxis with cyclosporine plus shot-term methotrexate except 3 with cyclosporine alone. Variables considered in statistical analyses were age, gender, diagnosis, disease risk, conditioning, year of transplant, total nucleated and total CD34 positive cell dose, positivity of CMV, sex, ABO and HLA mismatches. The impact of acute GVHD was assessed as a time-dependent covariate using Cox models.
Results: Median follow up period of survivors (N=97) was 83 (8-157) months after transplant. Cumulative incidence of grade II-IV and III-IV AGVHD at 100 days after CBT was 66 (95% confidence interval [CI], 58-74) and 10 (95% CI, 5-15)%, respectively. Only 20% of patients developing grade II AGVHD required steroid. In multivariate analysis, patients with grade II AGVHD showed tendency toward lower incidence of relapse (16% *vs* 30% at 5 years) and better disease-free survival (DFS: 75% *vs* 61% at 5 years) compared to grade 0-I AGVHD. However, patients with grade II AGVHD showed significantly lower incidence of relapse (8% *vs* 41% at 5 years, p<0.01) with no increase of TRM (3% *vs* 0% at 5 years, N.S.), leading to higher DFS (88% *vs* 55% at 5 years, p=0.03) compared to grade 0-I AGVHD among patients with acute leukemia in CR1 or CR2.
Conclusion: It is suggested that patients developing grade II AGVHD had better survival due to enhanced graft-versus leukemia effect after single unit CBT. It is the first analysis evaluating the impact of grade II AGVHD on better survival.

**P578**

**Leukaemia escape from HLA-specific T-lymphocyte pressure in a recipient of HLA one locus-mismatched BMT**

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The mechanisms of leukemia relapse in HLA-mismatched HSCT recipients remain largely uninvestigated. A case of leukemia escape from an HLA-specific cytotoxic T lymphocyte (CTL) response in a recipient of HLA one locus-mismatched BMT is presented. A 24-year-old man with primary refractory T lymphoblastic leukemia/lymphoma received BMT from his HLA-B*51:01-mismatched mother. The patient developed acute GVHD on day 46 (maximum grade: III on day 53), which was incurable and transitioned to chronic GVHD. The patient relapsed on day 261 and died on day 279. Flow cytometric analysis for HLA-A, B, and DR showed that only the expression of B51 was down-regulated in post-transplant leukemia blasts compared with that in pre-transplant blasts. Ten CTL clones were isolated from the patient's blood when acute GVHD developed. The nucleotide sequences of the uniquely rearranged TCR Vbeta gene of each clone indicated that 10 clones had been derived from six independent clones. All six independent clones lysed B51-positive recipient EB virus-transformed lymphoblastoid cells (B-LCL) and B*51:01 cDNA-transfected donor B-LCL, but failed to lyse B51-negative donor B-LCL (Cr release assay). COS cells transfected with B*51:01 cDNA alone clearly stimulated IFN-gamma production by six CTL clones. These data indicate that all clones recognized the B*51:01 molecule as an alloantigen and suggest that the CTL response toward the B*51:01 molecule accounted for a majority of the recipient's CTL alloresponse during acute GVHD. The pre-transplant leukemia blasts were lysed by CTL clones, whereas the post-transplant leukemia blasts were not lysed by any CTL clones. The IFN-gamma ELISPOT assay revealed that B*51:01-reactive T lymphocytes in patient blood on day 232, one month before

clinical leukemia relapse, were detected at a level nearly equal to the level of recipient B-LCL-reactive T lymphocytes, that is, the total alloreactive T lymphocytes. Taken together, these results suggest that CTLs specific for the HLA-B*51:01 molecule were generated in the patient blood during acute GVHD, and these CTLs continued to produce immunological pressure on leukemia blasts for at least eight months after transplantation, but B*51:01-down-regulated leukemia blasts escaped from the pressure of B*51:01-specific CTLs, and the leukemia clinically relapsed. These findings can explain, at least in part, the mechanism of how leukemia relapse occurs during persistent GVHD after HLA-mismatched HSCT.

**P579**

**Bath and cream PUVA treatment in acute cutaneous GvHD**

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Background: High dose glucocorticoids (prednisone at 1–2 mg/kg) have been considered the standard initial treatment for acute graft-versus-host-disease (aGVHD). Major drawbacks of systemic steroid therapy are acute and delayed complications, in particular, the increased risk of infections. Systemic PUVA has been shown to be effective as a secondary treatment in cutaneous GVHD. To our knowledge no studies have performed where methoxalen was applied topically (bath and cream PUVA).
Objective: To evaluate the efficacy of bath and cream PUVA therapy initiated either to control cutaneous aGVHD in pts resistant to standard steroid therapy or to accelerate steroid reduction.
Methods: Charts of pts who underwent PUVA therapy during 2010 and 2011 were evaluated. The efficacy was defined from either a reduction of the steroid dose and/or a clinical improvement of the cutaneous GvHD (complete response (CR), partial response (PR): reduction of at least 1 stage).
Results: 19 pts (2 stage I, 6 stage II, 9 stage III, 2 stage IV) underwent PUVA therapy (13 pts (68%) bath PUVA, 6 pts (42%) cream PUVA). One pt was excluded from the evaluation. The mean number of treatments was 19,6 (25,8 for bath and 15,0 for cream PUVA). The mean cumulative UVA dosage was 56,7 J/cm$^2$ (bath PUVA) and 41,2 J/cm$^2$ (cream PUVA) respectively. 62% of the patients showed a CR and 38% a PR. In 73% of the cases a reduction of the steroids was enabled. The mean steroid reduction was 64%.
Conclusion: Bath and cream PUVA was effective, well-tolerated and may be an alternative in pts with cutaneous aGvHD in whom systemic PUVA is not appropriate.

## Stem Cell Research and Experimental Stem Cell Transplantation

**P580**

**Suppression of self-renewal and repopulation of AML1/ETO and PLZF/RAR-alpha-positive haematopoietic stem cells by the deacetylase inhibitors dacinostat and vorinostat**

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In acute myeloid leukemia, the most primitive population of leukemic stem and progenitor cells (LSC) are thought to account for relapses of the disease. Deacetylase inhibitors (DACi) induce growth inhibition, cell cycle arrest, premature senescence and apoptosis of malignant cells and may therefore be used as a maintenance therapy. Aim of our study was to analyse the impact of various DACi on 1) the proliferation and self-renewal capacity of LSC and 2) DACi-mediated gene regulation.
Methods: Murine Sca1+/lin- hematopoietic stem cells (HSC) were retrovirally infected with AML1/ETO exon9 and PLZF/RAR-alpha, both known to induce an acute leukemia in C57BL/6N mice, and assesed by serial replating and day 12 spleen colony-forming unit (CFU-S) assays. For gene expression studies, AML1/ETO exon9- and PLZF/RAR-alpha-transduced 32D cells were analysed by Western blot after treatment with valproic acid (VPA, 150 µg/ml), dacinostat (2.5-20 nM) or vorinostat (1 or 2 µM).
Results: AML1/ETO or PLZF/RAR-alpha-positive Sca1+/lin- HSC had a serial replating capacity far exceeding that of mock infected controls. Prolonged treatment with dacinostat, vorinostat or VPA resulted in a progressive loss of colony forming cells after the second plating suggesting exhaustion of leukemic progenitors. Western blot analysis of AML1-ETO- and PLZF-RAR-alpha-transduced 32D cells showed a concentration-dependent downregulation of Bmi-1 and c-myc after 48 hours of incubation with dacinostat, vorinostat and VPA which may have contributed to vanishing of leukemic progenitors. The repopulation capacity of AML1/ETO- or PLZF/RAR-alpha- positive Sca1+/lin- HSC was analysed by injecting all the progeny grown within a 7-day culture period in presence of cytokines± DACi. Dacinostat- and vorinostat-treated cells gave rise to a significantly lower number of spleen colonies compared to VPA-treated cells or control cultures. Engraftment of LSC in the spleen was determined by qPCR showing a deleterious effect of dacinostat, as we failed to detect an AML1/ETO- or PLZF/RAR-alpha signal. Vorinostat gave conflicting results with reduction of AML1/ETO- and complete deletion of PLZF/RAR-alpha-positive cells, resp., whereas VPA had no or just a minor inhibiting effect.
Conclusion: We here demonstrate efficious inhibition of proliferation, self-renewal and engraftment of AML1/ETO and PLZF/RAR-alpha-positive stem and progenitor cells by dacinostat and vorinostat. DACi treatment was associated with reduced protein levels of Bmi-1 and c-myc.

**P581**

**Examining the cross-talk between breast cancer tumour-initiating cells and bone marrow environment to target bone metastases**

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Metastatic dissemination is a major cause of cancer-associated death. In breast cancer, bone, lung and brain are the main sites of tumor relapse. The bone marrow (BM) is a reservoir for metastatic breast cancer cells that can enter into a quiescent state and remain latent for decades after dissemination. By still unknown mechanisms, dormant tumor cells can reacquire proliferative ability, causing osteolytic or osteoblastic lesions.
We have investigated how breast cancer cells affect the BM stroma when they home to an endosteal niche and how the BM microenvironment can influence properties of tumor cells. We have taken advantage of the human adenocarcinoma derived cell line, MDA-MB231 subclone SCP1833, which has a high propensity for bone metastases following intracardiac injection. Bioluminescence imaging followed tumor cell dissemination and cell sorting isolated different cell populations from the BM environment. Excluding cells of the hematopoietic and erythroid lineages (CD45+TR119+) from the analysis, we sorted endothelial cells (CD31+), osteoblasts (Sca1-CD31-CD51+) and mesenchymal progenitors (CD31-Sca-1+CD51+) from tumor-bearing and naive mice and compared BM stromal populations by a genome wide-transcriptome study. Microarray analysis revealed that the BM is strongly affected by breast cancer cell dissemination. Several components of key molecular pathways involved in tumor development (including TGFBR, PDGFRB, EGFR, HGFR and IGFR signaling pathways) are modulated in

the BM of mice with tumor cells. PIK3CA, a downstream effecter of several of the above-mentioned pathways, is up-regulated in the BM stroma of tumor-bearing mice. We are following up this analysis by testing the effects of the PI3K-mTOR inhibitor BEZ-235 in a model of breast cancer bone metastases. We want to elucidate how BEZ-235 influences survival and proliferation of tumor cells and how the BM responds to PI3K pathway inhibition.
To investigate the effect of BM environment on disseminated tumor cells, we compared the expression profile of SCP1833 primary tumors isolated from the mammary fat pad with metastatic tumor cells from the BM. We uncovered transcripts for surface proteins whose expression is down-regulated in cancer cells that have homed to the BM, compared to tumor cells growing in the mammary fad pad. These and other results will be presented to show the important role that the environment plays in the tumor cell transcriptome and ultimately in the response to therapy.

**P582**

**Mesenchymal stem cell progenitor content in donor bone marrow harvest might be predictive of a better outcome following bone marrow transplantation**

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Introduction: Mesenchymal stem cells (MSCs) are multipotent stem cells that are easily isolated from bone marrow (BM) and have a important role in supporting haemopoiesis for their bystander and immunomodulant proprieties. We investigated the outcome of patients underwent to allogenic BM transplantion on the basis of the MSC characteristics isolated from BM samples used for the transplantation.
Materials and Methods: We isolated MSCs from directly plated BM samples and cultivated for 3-4 passages in an MSC medium containing 10% foetal calf serum. We analysed MSCs for: i) the number of fibroblast colony forming units (CFU-F); ii) growth rates at the first passage (defined as the ratio: detached/ plated cell numbers) and the cumulative population doubling, iii) immunophenotype (CD45,34,14,90,73,105,146). We then stratified these results according to their median values and analyzed the number of TNC/kg, CD 34+ cells %, CD34+ cells/kg, LTC-IC/kg, CFU-GM/Kg, BFU-E/kg, the PMN and PLT engraftment, and the presence of GvHD. The t test and Chi square test were performed as statistical analyses.
Results: We isolated MSCs from 39 donors with a median of age of 27 years (18 pts >18 years and 8 <18 years). The P1 rate (a ratio between the cells detached at the first passage and those plated at the moment of harvesting) could represent an index of the number of MSC progenitors in BM grafts. We stratified data on the basis of the median P1 rate (0.124) and observed an inverse correlation of the number of detached cells at the first passage with the number of TNC/Kg ($P<0.05$) and the number of CD34+ cells, the LTC-IC, CFU-GM and BFU-E. PMN engraftment was faster in the samples with a P1 rate >0.124. Except for the CD146 expression no other differences were observed at the immunophenotype analysis. Although no significant data were shown in GvHD occurrence, it is important to note that patients treated with the samples containing MSC with a high expression of CD146 at P1 did not develop chronic GvHD. We also observed that the samples with more CFU-F/kg (>140 CFU-F/kg) were also the samples with more TNC/Kg ($P<0.05$) and the number of CD34+ cells /kg but the CFU-F analysis was only carried out on a few samples.
Conclusion: Our data suggest that the MSC content in the BM might be predictive of a faster PMN engraftment . These data need larger studies to confirm the importance of MSCs content within the BM grafts to on outcome after BM transplantation.

**P583**

***In vitro* expansion of umbilical cord mesenchymal stem cells: a comparison among different serum-free culture media**

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Background: Increasing interest is currently set in clinical applications of human mesenchymal stem cells derived from Wharton's jelly (UCMSC) because of potential off-the-shelf availability at cord blood banks. Foetal bovine serum (FBS) is routinely used for the expansion and differentiation of MSC; however such addictives represent a potential hazard in clinical applications, due to the risk of transmitting viral/prion disease and/or inducing xenogenic immune reactions in the recipient. Therefore we compared the efficacy of two recently marketed serum-free media with a "classical" medium (alpha-MEM containing 20% FBS).
Materials and Methods: MSCs were isolated from 12 human umbilical cords. Wharton's jelly was separated from the cord vessels and placed in three different 6-wells dishes containing: StemPro MSC SFM medium® (medium A) (GIBCO, Invitrogen), StemPro MSC SFM XenoFree medium® (medium B)-(GIBCO, Invitrogen), or alpha-MEM containing 20% FBS (medium C). Fresh medium was added twice a week up to 90% confluence. MSC were then harvested and re-plated at 8,000 cells/cm$^2$ for five passages Doubling Time (DT) values were calculated according to established formulas. Osteogenic, chondrogenic and adipogenic differentiations were performed according to standard protocols and confirmed by cytochemistry.
Results: MSCs isolated in the three media were morphologically similar and showed the same differentiation potential. No significantly differences were observed between DT in all media after the primoculture (P0); however classical alfaMEM medium allows significantly better expansion than A (passages P1-P3) and B (P2-P3) media (Table 1). It may be noted that Medium B has a generally lower DT than medium A, though the difference does not reach statistical significance.
Conclusion: Our observations suggest that serum-free expansion can be achieved for UCMSC, although at a lesser extent than using FCS-supplemented media. Medium A and B can be used interchangeably for *ex vivo* UCMSC expansion process.

**[P583]**

**Table 1. Meandoubling time (12 cultures for each medium) of cells grown in different media (column 1-3), and results of pairwise t-tests**

| | A | B | C | p A vs B | p A vs C | p B vs C |
|---|---|---|---|---|---|---|
| Passage 0 | 81,88 | 70,71 | 80,76 | n.s. | n.s. | n.s. |
| Passage 1 | 63,13 | 47,02 | 34,96 | n.s. | <0.01 | n.s. |
| Passage 2 | 64,41 | 59,21 | 37,83 | n.s. | <0.01 | <0.05 |
| Passage 3 | 136,27 | 123,77 | 64,34 | n.s. | <0.005 | <0.01 |

**P584**

**Increased levels of bone marrow endothelial cells, progenitor and mature, in childhood acute lymphoblastic leukaemia**

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The role of endothelial progenitors(EPC) and mature cells(EC) in angiogenesis of malignant diseases has attained the interest of many recent studies.
Aim: The immunophenotypic characterization of EPC and EC subpopulations in the bone marrow (BM) of children with acute lymphoblastic leukemia (ALL) at diagnosis and during treatment, as well as solid tumors (ST) without BM involvement. Methods: BM cells from children with ALL at diagnosis (ALLd, n=10), at day 15 (ALL15d, n=7), day 33 of treatment when remission is achieved (ALL33d, n=9), under consolidation therapy (ALLct, n=14), following the end of treatment (ALLet, n=13) and ST at diagnosis (ST, n=10) were studied. The putative antigenic phenotypes of EPC and EC were assessed using a 4-color flow cytometry in both the CD45negative and CD45negative/dim cell subpopulation.
Results: The highest levels of EPC subpopulation CD45neg CD133+CD34+VEGFR-2+ were estimated at diagnosis of ALL with statistically significant differences compared with ALL33d (p=0.024) and ALLet (p=0.012).
The more mature EC subpopulation CD45negCD31+CD34+ VEGFR-2+ was estimated to be highest in the group of ST, followed by ALL at diagnosis and ALL15d. Remission of ALL was associated with the lowest levels of this EC subpopulation. Statistical analysis revealed significant differences between ST *vs* ALL33d (p=0.025), ALLd *vs* ALL33d (p=0.011), ALL15d *vs* ALL33d (p=0.002). Statistical significant differences were also estimated when ALL33d was compared to ALL under consolidation treatment (p=0.037) and following the end of treatment (p=0.008). Another mature EC subpopulation, the CD45negCD146+CD34+VEGFR-2+, was also found to be highest at diagnosis of ALL. The same tendency was found with the double combinations CD45negCD31+VEGFR-2+, CD45negCD146+VEGFR-2+, CD45negCD133+VEGFR-2+ and CD45negCD34+VEGFR-2+.
In the CD45neg/dim cell subpopulation, the CD133+CD34+ VEGFR-2+ phenotype was significantly higher in ALL15d *vs* ALL33d (p=0.036) and ALLd *vs* ST (p=0.008). In the CD45neg/dim subpopulation the triple combination indicative of a more mature EC subpopulation CD31+CD34+VEGFR-2+ cells was higher in the group of ST at diagnosis.
Conclusion: The levels of both progenitor and more mature EC subpopulations seem to be highest at diagnosis of ALL. The most mature EC subpopulation tends to be higher in the group of solid tumors without BM involvement. The precise role of these findings warrant further investigation.

**P585**

**Differential effect of autologous versus allogeneic mesenchymal stem cell transplantation in lupus-prone mice**

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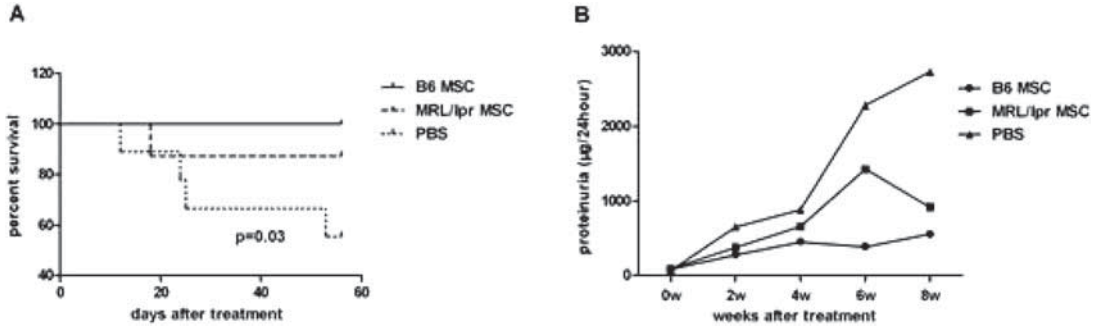
Objectives: Lupus is a systemic autoimmune disease characterized by the production of autoantibodies and end organ damage from immune complex deposition. Mesenchymal stem cells (MSC) possess immunomodulatory properties and also participate in tissue repair. Defects in MSC function are reported in cells derived from lupus mice and lupus patients. A key question in the use of MSC in autoimmune diseases is whether MSC from lupus donors are defective and thus not suitable for autologous transplantation.
Methods: MRL/lpr mice were treated with intravenous injection of $1\times10^6$ C57BL/6J (B6) MSC (n=8), MRL/lpr MSC (n=8), or phosphate buffered saline (PBS) (n=10) at 17 to 20 weeks of age at during active disease. All mice were sacrificed at 8 weeks after treatment and bone marrow and kidneys were harvested. Serum anti-dsDNA antibody and proteinuria were assessed by enzyme-linked immunosorbent assay. Bone marrow plasma cells were characterized by flow cytometry as identified by surface CD138 and intracellular IgG expression. Kidney pathological slides were stained with haematoxylin and eosin and scored in a blinded fashion. The average intensity of glomerular immune complex deposition in five independent fields of one kidney section per animal was quantitated.
Results: MSC transplanted from B6 donors, but not MRL/lpr donors, significantly enhanced mice survival compared to PBS injected animals (p=0.03) (Figure A). B6-derived MSC significantly decreased 24 hour proteinuria at 8 weeks after treatment compared to control, but MRL/lpr-derived MSC did not (Figure B. p<0.05). Neither source of MSC impacted serum anti-dsDNA antibody levels. B6-derived MSC-treated animals, but not MRL/lpr-derived MSC treated animals, had significantly fewer plasma cells in bone marrow and reduced glomerular IgG deposits compared to control (p<0.05). MSC administration did not significantly impact the overall renal pathology scores, though there was a trend towards lower scores in the B6 group versus the other two.
Conclusion: B6-derived MSC significantly improved MRL/lpr survival when given at peak disease activity. B6-derived MSC transplantation led to less proteinuria, decreased bone marrow plasma cells, and reduced glomerular IgG deposits. These results indicate MSC transplantation at time of peak disease does impact disease and suggest that allogeneic sources of MSC may be preferred to autologous sources.

**[P585]**



**P586**

**Case report: *in vivo* administration of mesenchymal stromal cells in a patient with graft-versus-host disease does not inhibit cytomegalovirus-specific T-cell response**

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Introduction: Mesenchymal stem cells (MSCs) are emerging as a helpful therapeutic tool for the treatment of Graft versus-Host Disease (GvHD). However, very little is known about the specificity of immunosuppression by MSC and the effect on cell-mediated immunity to pathogens causing post-transplant opportunistic infections. We report the immunological monitoring of a patient, receiving multiple doses of MSCs to treat post-transplant steroid-refractory GvHD, who presented Cytomegalovirus (CMV) reactivation the day after the first MSC infusion.

Method: To analyzed the patient's response to MSC infusions we studied two GvHD plasmatic markers, by ELISA assays, before MSC infusion and at day 7, 14 and 28 after cell therapy. Moreover, trying to understand the *in vivo* effect of MSCs on CMV-specific cell-mediated immunity, we evaluated the plasma concentration of IFN-gamma (ELISA assay) and the frequency of CMV-specific IFN-gamma secreting cells (ELISPOT assay) circulating in the peripheral blood (PB) of the patient before and after CMV infection.

Results: The patient, who presented at the moment of MSC infusion overlap cGvHD grade III, with skin and gut involvement, partially responded to the therapy, as clinically evaluated by the temporary GvHD reduction of one grade in the overall GvHD scoring at day 7/14 after MSC infusions. In accordance with clinical observations, we observed a significant decrease of two validated GvHD plasma markers TNFRI and IL2Ralpha (Paczesny S *et al.* Blood 2009) after MSC administration. In particular, we observed a 3.6 fold decrease of the TNFRI and a 2.9 fold decrease of the IL2Ralpha plasma concentrations at day 14 after MSC infusion, compared to the pre-MSC levels. Importantly, along with the appearance of high numbers of CMV DNA copies in the peripheral blood the day after the first MSC infusion, the patient showed an increase of IFN-gamma in the PB. Moreover, the frequency of CMV-specific mononuclear cells producing IFN-gamma following *in vitro* stimulation with a pool of CMV-derived, pp65 pp150 and IE1-specific peptides, increased 18 times compared to the pre-MSC values.

Conclusions: In summary, the monitoring of the patient suggests that MSCs, upon infusion, are able to exert an immunosuppressive effect on alloreactive GvHD-promoting cells without impairing the correct mounting of an anti-viral immune response.

**P587**

**Allogenic mesenchymal stem cell transplantation ameliorates nephritis in lupus mice via inhibition of B-cell activation**

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Objective: Recent evidence indicates that bone marrow-derived mesenchymal stem cells (BM-MSCs) possess immunosuppressive properties both *in vitro* and *in vivo*. We have previously demonstrated that transplantation of human MSCs can significantly improve the autoimmune conditions in MRL/lpr mice. The current study aimed to determine the mechanisms by which murine BM-MSC transplantation (MSCT) ameliorates nephritis in MRL/lpr mice.

Methods: 18-week-old MRL/lpr mice were treated with BM-MSCs derived from BAL b/c or saline. Mice were monitored for 24h proteinuria. Sera were tested for anti- dsDNA antibodies, BAFF levels, and levels of IFN-gamma, IL-2, IL-4, IL-10 and TGF-beta. After sacrificed at 26 weeks, kidneys were subjected to histologic examination. The percentage and number of marginal zone (MZ), T1 and T2 B cells in spleen were detected by flow cytometry.

Results: In this study, we found that MSCT can significantly prolong the survival of MRL/lpr mice. Eight weeks after transplantation, MSCT treated mice showed significantly smaller spleens than control animals, with fewer MZ, T1, T2, activated B cells and plasma cells. Moreover, serum levels of BAFF and IL-10 in MSCT-treated mice decreased significantly compared to those in the control group, while levels of serum TGF-beta were increased. Notably, decreased BAFF expression in both spleen and kidney was accompanied by decreased production of anti-dsDNA autoantibodies and proteinuria in MSCT-treated mice.

Conclusion: Our findings suggest that MSCT may suppress the excessive activation of B cells via inhibiting BAFF production in MRL/lpr mice.

**P588**

**High oxidation status induced the rearrangement of F-actin cytoskeleton of bone marrow-derived mesenchymal stem cells in patients with systemic lupus erythematosus via downregulation of RhoA signalling pathway**

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Objective: Systemic lupus erythematosus (SLE) is described as a hematopoietic stem cell disorder and bone marrow-derived mesenchymal stem cells (BMMSCs) from SLE patients is proved to be abnormal in their functions. This study was undertaken to determine the effect of oxidation status on F-actin cytoskeleton and RhoA signaling pathway in BMMSCs from SLE patients.

Methods: The F-actin cytoskeleton was observed by fluorescence microscopy after staining with Alexa Fluor 594 phalloidin. Transwell system was performed to determine the migration ratio of MSCs. RT-PCR and western blot were carried out to detect changes of RhoA signaling pathway. Serum level of SOD-1 was examined by ELISA and ROS level of MSCs was tested by flow cytometry. In order to investigate the effect of oxidation status on F-actin cytoskeleton of MSCs, 20 μM H2O2 was added into the growth medium of normal MSCs 1h before testing and 0.2 mM NAC, an antioxidant, was added into the growth medium of SLE MSCs.

Results: F-actin cytoskeleton of MSCs was rearranged in SLE patients. The percentage of abnormal MSCs was significantly higher in SLE patients than that of normal controls and increased as they were passaged. The migration capacity was also impaired as passaged. Both of the mRNA level and protein expressions of RhoA, which participates in a main regulatory signaling pathway of F-actin cytoskeleton and cell migration, were downregulated in SLE MSCs. Reduced serum SOD-1 level and higher intracellular ROS level suggested high oxidation status in SLE patients. H2O2, as an exogenous oxidant, induced upregulation of ROS level in normal MSCs. As a result, the F-actin cytoskeleton was rearranged and subsequently reduced migration ratio. While NAC, which downregulated ROS level, reversed the rearrangement of F-actin cytoskeleton and the impairment of migration capacity of SLE MSCs. RhoA expression was downregulated by H2O2 while upregulated by NAC.

Conclusion: These experimental findings suggest that the rearrangement of F-actin cytoskeleton of MSCs in SLE patients leads to the impairment of their migration capacity, which may result from the high oxidation status via downregulation of RhoA.

**P589**

**Activated NF-kB in mesenchymal stem cells from SLE patients inhibits osteogenic differentiation through down-regulating Smad signalling**

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Objectives: The osteoporosis of patients with systemic lupus erythematosus (SLE) is thought to be the results of over-osteoclastogenesis induced by pro-inflammatory cytokines such as TNF-alpha. However, the osteoblastogenesis in SLE patients is unclear. We investigated the bone morphogeneic protein (BMP)2 induced osteoblastic capacity of bone marrow-derived mesenchymal stem cells (BMMSCs) from SLE patients and the TNF signaling system in determining BMP-2 induced the regulatory pathways.
Methods: BMMSCs were differentiated into osteoblasts *in vitro* stimulated by BMP-2 and the osteogenic capacity was quantitated. cDNA microarray was performed between BMMSCs from four SLE patients and four healthy controls, and differentially expressed genes in BMP/TGF-beta signaling pathway were analyzed. The activation of BMP/Smad and NF-kappaB signaling pathways in SLE-BMMSCs were evaluated by detecting the phosphorylated Smad1/5/8 (p Smad1/5/8) and I-kappaB. TNF-alpha awas added and its effect on the activation of pSmad1/5/8 and BMP-2-induced osteogenic differentiation of normal BMMSCs was determined, while PDTC, a NF-kappaB inhibitor, was used to examine its effect on that of SLE-BMMSCs. Finally, BMP-2 level in the serum from SLE patients were detected by ELISA.
Results: The capacity of osteogenic differentiation of BMMSCs from SLE patients reduced as compared with that from healthy controls. The NF-kappaB signaling was active, while the BMP/Smad pathway was repressed in the BMMSCs from SLE patients. TNF-alpha activated NF-kB pathway and inhibited the phosphorylation of Smad 1/5/8 and BMP-2-induced osteoblastic differentiation in normal BMMSCs, while addition of PDTC to SLE-BMMSCs could partially restore them. The BMP-2 level was lower in the serum of SLE patients.
Conclusions: Our findings suggest that the activated NF-kappaB pathway in SLE-BMMSCs inhibits the BMP-2-induced osteoblastic differentiation through BMP/Smad signaling pathway. The inadequate osteoblastic differentiation may participate in the pathology of osteoporosis in SLE patients.

**P590**

**The application of human mesenchymal stem cells in clinical practice**

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Objectives: Mesenchymal stem cells (MSCs) have generated a lot of excitement in the field of regenerative medicine over the last decade due to their potential to differentiate into a variety of cell types, their ability to stimulate proliferation and differentiation of resident progenitor cells, and promote recovery of damaged tissues through secretion of a variety of cytokines and chemokines. MSCs can be used for the prevention and treatment of graft-versus-host disease (GvHD) in hematopoietic stem cell transplantation (HSCT) and hepatic regeneration in cases of cirrhosis.
Aim: The aim of the study was to present data on the use of human MSCs in clinical practice.
Methods: MSCs were taken from bone marrow (BM) and placenta (PLA) and expanded for 30–50 days *in vitro*. All samples were subjected to bacteriological and viral control. The MSCs' immunophenotypes were typical for cells of mesenchymal origin. Biological safety was evaluated by karyotyping in passages 4–5 and 9–10. The derived MSCs were presented in the native or frozen form. After protocol approval by the ethics committee, 30 child patients (4 with ALL, 4 AML, 1 biphenotypic acute leukemia, 4 Fanconi's anemia, 3 aplastic anemia, 1 adrenoleukodystrophy, 1 Wiskott-Aldrich syndrome, 1 SCID, 3 lymphohistiocytosis, 2 CML, 5 MDS and 1 osteopetrosis) and 10 adult patients (4 with chronic HBV-infection related cirrhosis and 6 with primary biliary cirrhosis) participated in the study.
Results: The indications for co-transplantation of MSCs from BM (99 doses) are: transplantation of HSC (in order to improve HSC engraftment and prevent GvHD) – 14, chronic GvHD – 2, acute GvHD – 10, and hypofunction of HSC transplants – 4 cases. In all cases allogeneic MSCs from BM were used, including 5 cases from relative donors (parent/sibling). The MSCs doses were 2; 1; 0.8 and 5.6 million per kg in 92; 5; 1 and 2 cases respectively. 9; 9; 4; 3; 1; 1; 2 and 1 patients received 1; 2; 3; 4; 6; 8; 11 and 12 doses of MSC transplants respectively. In all adult patients allogeneic MSCs from PLA (20 doses) were used for hepatic regeneration and hepatocyte differentiation. The PLA MSCs dose was 2.0 million per kg. All patients received 2 doses MSCs. Acute reactions during and after the PLA and BM MSC infusions were not observed.
Conclusions: Thus, the presented data reflects the possibility of the clinical application of allogeneic human MSCs from BM and PLA, harvested under strict quality and safety control.

**P591**

**Functional properties of human mesenchymal stem cells derived from multiple sclerosis patients as compared to those of healthy donors**

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Background: Mesenchymal stem cells (MSC) have been tried in the recent years, for the treatment of various experimental and human diseases. Our group has gained large experience with the application of treatment protocols involving the use of autologous hMSCs in neurological diseases, such as multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS).
Aim: To investigate whether MSCs obtained from MS patients are functionally equivalent to those of healthy individuals and to determine the effect of iron particles (Feridex©) on the functional properties of MSCs isolated from MS patients.
Methods: The neurotrophic effects, neural-differentiation potential and the immunological features of hMSC obtained from MS patients, were evaluated and compared to those of healthy donors. We used FACS analysis, co-culture of patients' MSCs with allogeneic lymphocytes and examination of culture supernatants for cytokine and chemokine production by cytometric beads assay. In addition, the effect of iron particles on the functional properties of the MSCs isolated from MS patients were evaluated using the above mentioned methods.
Results: MSCs obtained either from MS patients (MS-MSC) or healthy donors (HD-MSC) were equally able to differentiate into mesodermal cells, adipocytes and osteoblasts and to induce neural differentiation of PC12 cells, indicating that MSCs from MS patients preserve their neurotrophic properties. Both MS-MSC and HD-MSC secreted equal quantities of BDNF and NGF and could be similarly differentiated into neural-like and glial-like cells, upon induction with neurptrophic factors. MS-MSCs had the same suppressive effect on lymphocyte proliferation (85-90% inhibition using 3H-Thymidine incorporation). Both MS-MSC and HD-MSC strongly inhibited the secretion of IFNg ,TNFa, CCL5, CXCL10, and CXCL9 (>80%), by lymphocytes. An enhanced IL-10 and a trend toward increased IL-17 production was detected in lymphocytes co-cultured with HD- and MS-MSCs. Labeling hMSC with the iron particles did not result in any major effects or compromise of their functional properties.

Conclusion: Our findings show that hMSCs obtained from multiple sclerosis patients preserve their main functional properties and are largely similar to MSCs from healthy donors.

**P592**

**Raising the bar for stem cell transplant: feasibility and outcomes with SCT for patients over the age of 75 years**

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Introduction: SCT is a potentially curative procedure for a number of hematological malignancies. However, there remains limited data in the safety and outcomes with SCT for patients over age 75 years.
Methods: We performed a retrospective analysis for patients with relapsed hematologic malignancies aged ≥75 years that underwent SCT at UMass Memorial Medical Center since 2009.
Results: We identified 9 consecutive patients ≥75 years who underwent 10 SCT procedures. There were 7 males and 2 females. Diagnosis was multiple myeloma (n=4), acute myeloid leukemia (AML) (n=3) and non-Hodgkin lymphoma (n=2). Median age at original diagnosis was 74 years (range, 65-80), while median age at SCT was 77 years (range, 75-80). 8 SCTs were autologous (auto) and 2 were from allogeneic matched unrelated donors (MUD). Median number of chemotherapy cycles prior to SCT was 2 range (1-9) and median time from diagnosis to transplant was 19 months (range, 3.5–122). The 2 MUD SCTs were performed for AML with poor prognostic cytogenetics. One patient had received two prior auto-SCTs. Disease status prior to SCT was complete remission (CR) in 3, persistent disease in 6 and unknown in 1 patient. Mobilization chemotherapy for auto-SCT was cyclophosphamide in 6; etoposide/cytarabine in one and growth factor alone in one patient. Median number of CD34 cells infused was 5.0x10e6/kg range (2.8x10e6-6x10e6). Preparative regimen for auto-SCT were BEAM in 1, buslfan(Bu)/thiotepa in 1, and high dose melphalan (>140 mg/m$^2$ in 5) in 6 patients. The regimen for MUD-SCT was fludarabine/Bu (3.2 mgx2 or 3)/rabbit ATG. Graft versus host disease prophylaxis (gvhd) was tacrolimus/mycophenolate mofetil (MMF) and sirolimus/MMF. Median number of days hospitalized during SCT was 18.5 days (range, 12-28). Median time for neutrophil engraftment was 10.5 days (range, 9-18) and for platelet engraftment was 19 days (range, 14-49). All patients survived 100 days post SCT. Two patients have died at 6 and 9.6 months post SCT due to progressive disease. The two patients who underwent MUD SCT are disease free and without GVHD on minimal immunosuppression 8 and 10 months post SCT. The Kaplan-Meier survival estimate shows the probability of survival at 6 months to be 89% and 67% at 1 year (see Figure).
Conclusion: To our knowledge, this is the first analysis to evaluate the feasibility and outcome of SCT for patients over age of 75 years. SCT appears to be safe and effective in selected patients in this age group.

**P593**

**Analysis of the factors related with engraftment in allogeneic transplant patients**

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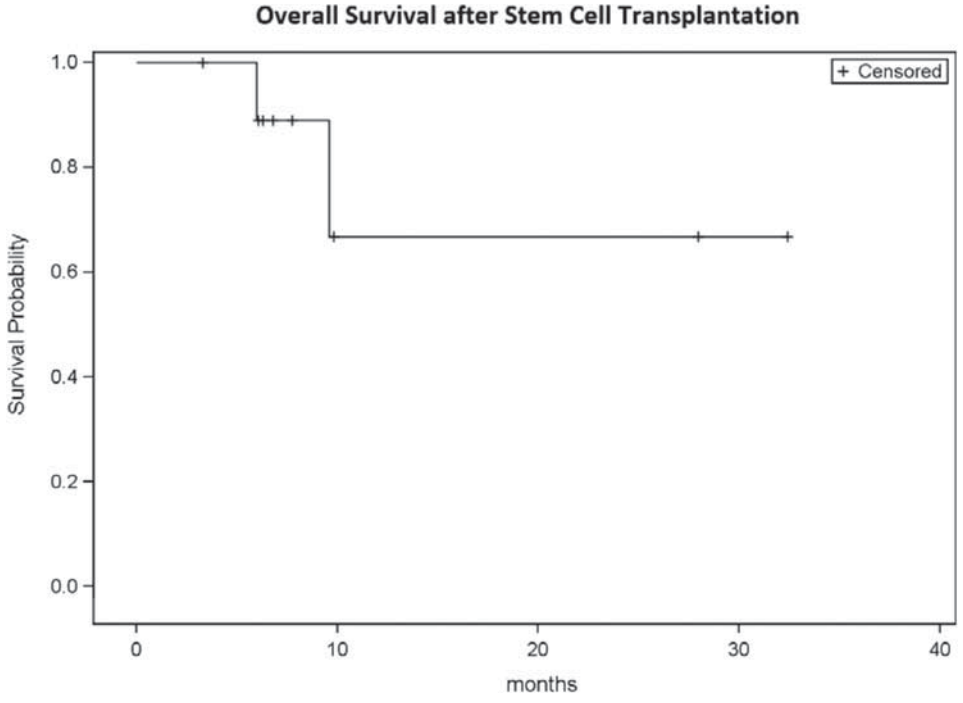
Introduction: Following the AHCST, engraftment occurs approximatelly between days 7-21.Two important complication of late engrafment are bleeding and infections.
Patient and Methods: A total 50 patients who underwent AHCST between July 2010-November 2010 were investigated retrospectively. In all patient who underwent AHCST with fullmatch donors the relation between engraftment development and diagnosis, recipient age, donor age, recipient gender, donor gender, blood group compatibility, amount of CD34+ given, conditioning regimen were investigated.
Results: The diagnosis of patient were 36 acute leukemia (AL) (72%), 5 aplastic anemia (AA) (%10), 3 myelofibrosis (MF) (%6), 3 non Hodgkin lyphoma (NHL) (%6), 3 (%6) other diseases (Hodgkin lyphoma (HL), sickle cell anemia (CCA) and myelodysplastic sydrome (MDS)). 21 of patients were female (%42) and 29 patients were male (%58). 30 (%60) of patients were given without total body irradiation (TBI) conditionig regimen,

**[P592]**



20 (%40) of patients were given containing total body irradiation (TBI) conditionig regimen. The median age of 50 patients was found 30.5 years (21-41). The median time of neutrophil engrafment was found 16,2±3,4 and median time of platelet engrafment was found 11,5±4,3. The patients performed TBI neutrophil engraftment median value 14,5(14.5-21) between the patients performed non TBI neutrophil engraftment median value 15(13-16,5) was found statistically significant (p=0,014). As the age of recipient neutrophil engraftment time is shortened. A negative relationship was found between recipient age and neutrophil engraftment time and this relationship was not statistically significant (p=0,186). There was a positive correlation between recipient age and platelet engrafment time but this was not statistically significant (p=0,547). There was no statistically significance between CD34+ count and neutrophil and platelet engraftment time (p=0,499, p=0,854, respectively. No significant relationship was found between neutrophil and platelet engraftment time and diagnosis, donor age, recipient gender, donor gender, blood group compatibility.
Conclusion: Conditioning regimen is an important factor for neutrophil engraftment time. Recipient age and CD34+ cell count may be important factors both neutrophil and platelet engraftment.

**P594**
**Validation of back-up cryopeservation process of haemopoietic progenitor cells**
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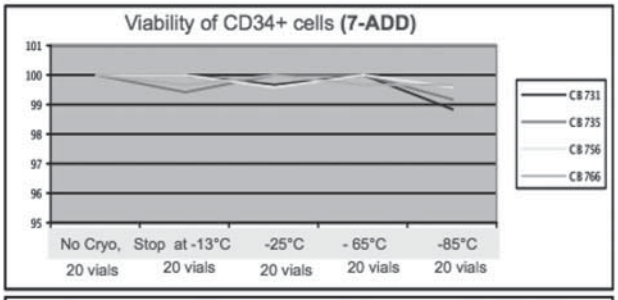
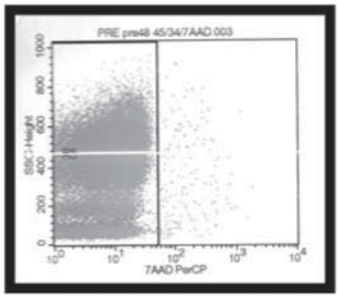
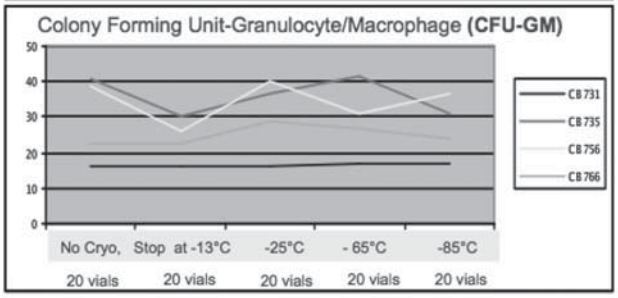
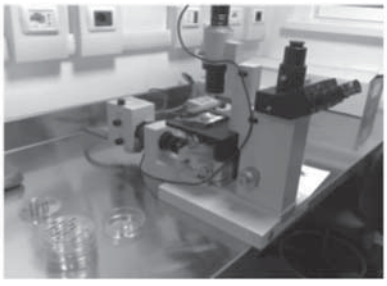
The establishment of process control is a primary objective of the Processing Facility QM Program. Hemopoietic progenitor cells shall be cryopreserved using controlled rate freezing, but a validated back-up plan is essential to resolve any adverse event during cryopreservation, to prevent loss of stem cells, and to maintain equivalent recovery and viability of nucleated cells.
We validated a back-up model of HPC cryopreservation procedure that can be used in case of failure of controlled rate freezing due to adverse event (eg. rupture or damage of liquid nitrogen freezer, loss of electric power…), when a second nitrogen freezer is not avalable as a back-up.
Method: We selected 4 cord blood units non suitable for clinical use (TNC, total nuclear cells <1200x10e6). Each cord blood unit was divided in 25 vials of 1 mL and tested for TNC, CD34+ cells, viability (7-AAD) and CFU-GM/BFU-E: 5 vials (x4=20) was analized without cryopreservation and 20 (x4=80) vials was cryopreserved using 10% DMSO (ALCHIMIA srl) in nitrogen vapour freezer NICOOL PLUS (AirLiquide). We simulated four different adverse event occurring during cryopreservation, due to interruption of controlled rate freezing at different temperatures: -13°C, -25°C, -65°C, -85°C (5 vials each conditions, each cord blood).
After interruption of controlled rate freezing, vials was immediatelly trasferred in mechanical freezer -80°C in a polystyrene box containing cotton wool, simulating a cryopreservation back-up procedure. 24 hours later vials was transferred in vapour fase nitrogen storage tank, simulating a standard long term storage.
In order to analized the biological and functional effect on HPC due to adverse event occurring during cryopreservation, each vial was thawed and tested for TNC, CD34+ cells, viability (7-AAD) and CFU-GM/BFU-E.
Results: For each cord blood in all different conditions analized, no significative differencies was documented on TNC, CD34+ cells, viability and CFU-GM/BFU-E.

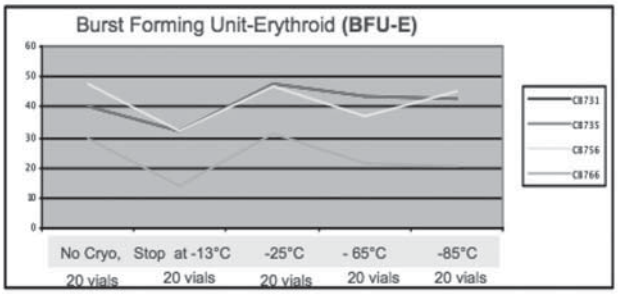
**[P594]**

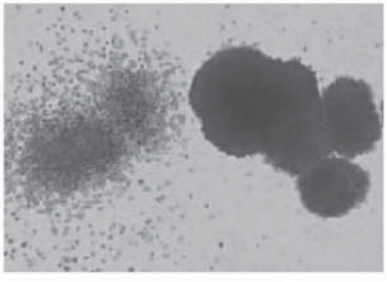




Conclusions. We validated a back-up cryopreservation procedure using a mechanical freezer -80°C, demonstrating that clonogenicity and functional activity of HPC is not affected even when cryopreservation is early interrupted, if vials is immediately transferred in a protective box containing cotton wool, in mechanical freezer -80°C, resolving an adverse event when cryopreservation is running. Storage of cryopreserved stem cells shall be in nitrogen liquid or vapour phase tank, as standard describes.

**P595**

**Intra-bone marrow transplantation of umbilical cord blood haematopoietic stem cell: a single-centre experience**

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Objectives: Umbilical cord blood (UCB) is an alternative source of hematopoietic stem cells in patients requiring allo-SCT. The advantages of UCB transplantation are: prompt availability of CB, absence of donor risk, tolerance of 1-2 antigen HLA mismatch and reduced incidence of severe acute GVHD (aGVHD). The major limiting factor is the low number of hematopoietic stem cells in a CB unit.
Methods: Eight patients (2 male and 6 female) were submitted to intra-bone UCB transplant. Median age was 43 years (range 26-64) and median body weight was 62 kg (range 40-90). The median number of nucleated cells infused was 2 x $10^7$/kg (range 1.2 x $10^7$-3.55 x $10^7$/kg) and the median number of CD34+ cells was 0.805 x $10^5$/kg (range 0.43 x $10^5$-1.1 x $10^5$/kg). The main characteristics are reported in Table 1.
Results: At the last follow up four patients were alive: three patients were in complete remission (CR). Primary graft failure occurred in one acute myeloid leukemia (AML) patient. Two patients died during aplastic phase for Gram negative sepsis (n=1) and Gram positive sepsis associate with multiple organ failure syndrome (n=1). One patient with refractory Hodgkin Lymphoma (HL) died on day +150 for progression disease. Refractory AML relapsed on day +180 and died on day +240.
The median time of neutrophil (>500/$mm^3$) and platelet (>20.000/$mm^3$) recovery was 29 and 38 days respectively. On day +90 mixed chimerism was present in three patients and complete donor chimerism in two patients. Last evaluation showed mixed chimerism in two patients (respectively on day +90 and on day +540) and complete donor chimerism in one patient (day +90).
Early transplant related complications were: bacterial sepsis (n=7), cytomegalovirus infection (n=6), pneumonia (n=3), primary graft failure (n=1), renal failure (n=1), Coombs positive hemolytic anemia (n=1), and calcineurin inhibitor related microangiopathy (n=1). Late transplant related complications were: spondylodiscitis (n=1) and Guillain Barre syndrome (n=1). No GVHD was documented in our cases.
Conclusion: Intra-bone UCB transplantation is a procedure potentially overcoming the issue of graft failure due to the low number of UCB nucleated cells. Our preliminary data suggest that it's a valid option for patients without HLA compatible donor requiring allo-SCT, as soon as possible, because of high risk of relapse. The mechanisms of UCB engraftment and the low incidence of GVHD require further studies.

**P596**

**Validation of analytical methods in GMP: a practical approach**

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The quality and safety of advanced therapy products must be maintained throughout their production and quality control (QC) cycle, ensuring their final use in the patient.
We validated three QC methods according to ICHQ2 Guidelines and EU Pharmacopoeia, to evaluate the cell count, the LAL

**[P595]**

Table 1. Patients' characteristics.

| Patient | Age | Sex | Disease | Phase | Gvhd prophylaxis | Conditioning regimen | HLA compatibility | Last follow up |
|---|---|---|---|---|---|---|---|---|
| L.G. | 65 | M | AML secondary to MDS | CR2 | Cyclosporin A (1 mg/kg) Mycophenolate (30 mg/kg) | Fludarabine (100 mg/sqm) Cyclophosphamide (100 mg/kg) Thiotepa (10 mg/kg) ATG (5 mg/kg) | 5/6 (mismatch locus B) | CR (day +630) |
| C.P. | 27 | M | HL | Refractory | Cyclosporin A (1 mg/kg) Mycophenolate (30 mg/kg) | Fludarabine (100 mg/sqm) Cyclophosphamide (100 mg/kg) Thiotepa (10 mg/kg) ATG (5 mg/kg) | 5/6 (mismatch locus B) | death (day +150) |
| S.C.C. | 27 | F | ALL | CR3 | Cyclosporin A (1 mg/kg) Mycophenolate (30 mg/kg) | Total body irradiation (12 Gy) Cyclophosphamide (120 mg/kg) ATG (5 mg/kg) | 5/6 (mismatch locus B) | death (day +30) |
| C.S. | 32 | F | ALL | Refractory | Cyclosporin A (1 mg/kg) | Fludarabine (100 mg/sqm) Cyclophosphamide (100 mg/kg) Thiotepa (10 mg/kg) ATG (5 mg/kg) | 5/6 (mismatch locus B) | death (day +10) |
| L.D.A.L | 43 | F | AML | CR2 | Cyclosporin A (1 mg/kg) Mycophenolate (30 mg/kg) | Busulfan (12.8 mg/kg) Cyclophosphamide (120 mg/kg) ATG (5 mg/kg) | 6/6 | graft failure (day +390) |
| A.T. | 55 | F | AML | Refractory | Cyclosporin A (1 mg/kg) Mycophenolate (30 mg/kg) | Fludarabine (100 mg/sqm) Cyclophosphamide (100 mg/kg) Thiotepa (10 mg/kg) ATG (5 mg/kg) | 5/6 (mismatch locus B) | death (day +240) |
| E.Z. | 54 | F | AML | CR1 | Cyclosporin A (1 mg/kg) Mycophenolate (30 mg/kg) | Fludarabine (100 mg/sqm) Cyclophosphamide (100 mg/kg) Thiotepa (10 mg/kg) ATG (5 mg/kg) | 5/6 (mismatch locus B) | CR (day +150) |
| L.K. | 44 | F | ALL | CR1 | Cyclosporin A (1 mg/kg) Mycophenolate (30 mg/kg) | Fludarabine (100 mg/sqm) Cyclophosphamide (100 mg/kg) Thiotepa (10 mg/kg) ATG (5 mg/kg) | 6/6 | CR (day +150) |

test and the immunophenotype, considering the tests' accuracy, precision, repeatability, specificity, detection limit, linearity, and range.
Materials and Methods: The cell count was performed by using the Fast Read 102® disposable count chamber. As the cell count is a potency test, we had to check: accuracy, precision, and linearity. Viability was checked using the Trypan Blue vital dye. To validate the endotoxin test we used a kinetic Chromogenic LAL test: a limit test for the control of impurities. We evaluated precision, specificity and detection limit on supernatant, on cell therapy products at different dilutions, and on pyrogen-free water as a negative control. The immunophenotype validation required a performance qualification of the FACS Canto II using two types of standard beads to check the cytometer reproducibly set up. As an identity test, we evaluated specificity by using the fluorescence minus one (FMO) method: each cell population was stained with all kinds of fluorochrome except that of interest. All the experiments were repeated thrice to test precision.
The data were statistically analyzed by means (M), standard deviation (DS) and coefficient of variation percentage (CV%) inter and intra operator.
Results: All the tests performed met the established acceptance criteria of CV% <10%. For the cell count, the precision reached by each operator had a CV% <10%. CV% of the cell viability in three different counts performed by the same operator was <5%. The best range of dilution, to obtain a slope line value very similar to 1, was between 1:10 and 1:50.
The LAL test performed thrice on the same samples is repeatable, and specific, as the percentage of spike recovery of each sample was between 0.25 UE/ml and 1 UE/ml with a CV% <10%. The coefficient of correlation ≤0.980 and CV% <10% of the standard curve tested in duplicate showed the test's linearity and a minimum detectable concentration of 0.005 EU/ml.
The CV% less than 10% obtained during immunophenotype validation confirmed the specificity of the test and intra and inter experiment precision.
Under our standard method procedures, these assays may be considered good QC methods for the batch release.

**P597**
**Development of experimental animal model to improve the efficacy of human gene therapy for X-CGD**
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Background: X-linked chronic granulomatous disease (X-CGD) is a primary immunodeficiency that mutations in gp91phox gene encoding component of NADPH enzyme system result to failure of production of microcidal reactive oxygen species (ROS) in granulocytes. Stem cell gene therapy is considered a promising therapy of disease. However, the existence of peripheral granulocytes with failure of functional reconstitution despite success of transduction of gp91phox gene has been a clinical problem. In previous, we demonstrated that transplanted hematopoietic stem/progenitor cells (HSPCs) from X-CGD mice transduced gp91phox gene clearly separated into two fractions of peripheral granulocytes: ROS productive or non-productive populations even the presence of transgene in ROS non-productive population. Based on this result, we attempted to develop of gene therapy model to resolve a clinical problem in human gene therapy of X-CGD.
Methods: BM KL (c-Kit+, lineage-) cells isolated from X-CGD mice were transduced with a retroviral vector harboring murine gp91phox cDNA together with Kusabira Orange (KO) gene. After culture, KL cells with KO dull intensities that mean minimum transduction of target gene were sorted. These cells were transplanted into lethally irradiated X-CGD mice. Functional reconstitution of peripheral granulocytes characterized by ROS production were assessed by APF dye staining assay in conjunction with KO fluorescence intensities in Gra-1+ population of recipient mice.
Results and Plan: The majority of reconstituted Gra-1+ cells from all 4 recipients were APFpositive-KObright population at 2 weeks after transplantation. However, these populations were clearly separated into 2 fractions: APFpositive-KObright and APFnegative-KOdull in 3 mice, and one recipient showed disappearance of APFpositive-KObright population at 4 weeks. Interestingly, one of three recipients showed gradually decrease of APFpositive-KObright population during an observation period. Thus, X-CGD mice showed gradual decrease of peripheral granulocytes achieved functional reconstitution even transplantation of KL cells transduecd target gene. This result seems to develop experimental model to resolve clinical problem in human gene therapy. We are studying several possibilities to explain this result: 1) low competitive repopulation activity or antigenicity of a transplanted cell of gene-transduced X-CGD HSPCs, or 2) conditions for expression of transgene or translation.

**P598**
**Enumeration of CD34+ cells: comparison between the observed and the theoretical coefficient of variation**
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Introduction: CD34 positive cells are considered to be rare events in peripheral blood stem cells (PBSC), since they represent less than 1% of the harvested cells by aphaeresis.Rare events statistics logically match a "Poisson" probability distribution.The aim of this study is to calculate the coefficient of variation (CV) of 30 CD34+ cells counts and then to compare them the theoretical CV according to the Poisson law.
Material and Methods: 30 PBSC products collected by cytapheresis were hazardously included in this study.Each sample was diluted with PBS (1:9) before staining with CD45 FITC, CD34 PE and 7'AAD according to the "lyse no wash" procedure.At least 105 events and/or 700 CD34+ cells were acquired using a EPICS XL cytometer (Beckman Coulter).The percentage of the CD34+ cells was calculated in triplicate, for each sample in a blinded manner, according to ISHAGE (International Society for Hematotherapy and Graft Engineering) gating strategy and using WinMDI2.9 software.The observed CV were calculated using the following formula CV=100*SD/m (SD: standard Deviation and m: mean of three counts).The theoretical CVs were calculated according to Poisson law as follows:
SD = r and CV% = 100SD/r = 100/ r, were SD: Standard Deviation and r: number of CD34+ cells.
The observed and theoretical CVs were compared using a simple linear regression plot.
Results: See Table.

**[P598]**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Theoretical CVs | 30 | 2,70 | 16,50 | 5,7800 | 3,33688 |
| Observed CVs | 30 | 0,40 | 43,30 | 6,8533 | 9,19812 |

Simple linear regression plot gave the following least squares equation by comparing the observed (y) to the theoretical CVs (x):y=2.46x+7.36 (r= 0.89)
Conclusion: We conclude that even if CD34+ cells are considered as rare events they do not match the theoretical concept of the Poisson law.

**P599**

**Cord blood-derived endothelial progenitor cells: a superior building company in the angiogenesis market**

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Objective: Endothelial progenitor cells (EPCs) are a promising tool in regenerative medicine. We designed a comparative study of peripheral (adult, PB) versus umbilical cord blood (CB) derived EPCs in order to investigate the angiogenic properties of these two sources.
Methods: Mononuclear cells (MNCs) were isolated from PB (n=4) and CB (<24 hours, n=4) and cultured on fibronectin-coated cell culture plates in endothelial cell culture medium until the formation of cobblestone-shaped colonies. Colony-derived cells were expanded for further investigation and their endothelial phenotype was confirmed evaluating the uptake of Di-I-acetylated-low-density lipoprotein (Ac-LDL) and expression of endothelial cell-surface antigens (CD34, CD31, CD146, vWF, KDR) and CD45. Proliferation rates of CB and PB derived EPCs were determined using the MTT assay. The angiogenic properties of colony-derived EPCs were assessed by *in vitro* capillary-like network formation assay: early-passage cells were seeded (20,000 cells per well) onto 96-wells plates coated with Matrigel. Capillary-like network formation and maintenance were evaluated with an inverted microscope after 4-18-24-48-72 hours of incubation; images were taken from 3 random fields of Matrigel wells to assess the number of branch points per field to quantify the degree of tubulogenesis. Each experiment was performed in triplicate.
Results: The median frequency of colonies obtained was higher in CB than PB (0.49/$10^7$MNCs *vs* 0.05/$10^7$MNCs) and CB-derived EPCs had higher proliferative potential than PB-derived EPCs. Both CB and PB colony-derived cells incorporated Ac-LDL, expressed the endothelial cell-surface antigens and were CD45-. Both CB and PB derived EPCs formed capillary-like structures in Matrigel, but CB-derived tubes formed earlier and more complex networks than PB-derived EPCs. Moreover, the number of branch points in CB-derived capillary-like networks was higher than PB-derived networks (16.3±4.3 *vs* 7.1±-2.6). Finally, CB-derived capillary-like networks were maintained for at least 72 hours, while PB-derived networks started to disassembly in 48 hours.
Conclusion: Our study confirms the different clonogenic and proliferative potential of EPCs derived from PB and CB and demonstrates a superior angiogenic potential of CB-derived cells. Our preliminary data indicate that CB-derived EPCs have a better angiogenic potential than PB-derived EPCs and CB is a promising source of EPCs for regenerative medicine purpose.

**P600**

**Preclinical analysis of immunosuppressive and cytotoxic effects of imatinib, nilotinib and dasatinib in combination with cyclosporine A or rapamycin**

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Introduction: Administration of tyrosine kinase inhibitors (TKI) post allogeneic stem cell transplantation (allo-SCT) may help prevent or treat relapse in Philadelphia positive leukaemia, however the feasibility and toxicity of this strategy is still under investigation. Besides their cytotoxic effects TKI have also been reported to be immunosuppressive. Application of TKI in the early post-transplant phase will often mean combined treatment with immunosuppressive agents like cyclosporine A (CSA), Tacrolimus or mTOR inhibitors [Rapamycin, (Rapa) or Everolimus].
Methods: We investigated the immunosuppressive and cytotoxic effects of Imatinib (Ima), Nilotinib (Nilo) and Dasatinib (Dasa) in combination with CSA or Rapa *in vitro*. T-cell stimulation was done with PHA, and Ki67 expression analysed after 72h by flow cytometry. Chromium-release assay was used to study effects on NK cell cytotoxicity. Drug toxicity on cell lines was analysed by flow cytometry after staining with 7AAD. Combination effects were analysed using Calcusyn® software.
Results: All TKI had dose-dependent cytotoxicity on K562 cells. Addition of CSA or Rapa did not alter cytotoxicity. All TKI had dose-dependent inhibition of T cell activation and NK cell cytotoxicity. Combination of TKI and CSA or Rapa showed synergistic inhibition of T cell activation with combination index 0.47 to 0.74 for Ima + CSA, 0.28 to 0.56 for Nilo + CSA, 0.55 to 0.85 for Dasa + CSA, 0.25 to 0.63 for Ima + Rapa, 0.10 to 0.29 for Nilo + Rapa and 0.50 to 0.68 for Dasa + Rapa.
A concentration of 5nM Dasa decreased CFU to 42% of control. Higher doses of Dasa were excluded to allow for analysis of drug combinations. Doses of other TKI were adjusted to 40nM Nilo and 120 nM Ima. Ima and Nilo reduced CFU to 59.8% and 91.8% respectively. CSA (200 ng/ml) and Rapa (0.5 nM) reduced CFU to 86.7% and 48.4% respectively. Addition of CSA to the TKI did not further reduce CFU while addition of Rapa to TKI strongly reduced CFU to 25.8%, 34% and 12.1%, for Ima, Nilo and Dasa respectively.
Conclusion: Our data show combination effects of TKI with CSA and Rapa which need to be taken into consideration when combining these drugs. Our results lay the ground work for further preclinical and clinical studies.

**P601**

**Alpha-2 integrins are marginally involved in the binding of activated human T lymphocytes to allogenic mesenchymal stromal cells *in vitro***

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Due to their ability to inhibit steroid-resistant graft-versus-host disease, mesenchymal stromal cells (MSC) are viewed as a potent therapeutic adjuvant to complement allogenic hematopoietic stem cell transplantation. However the mediators involved in alloreactive T cell inhibition by MSC are not fully identified, nor is the relative importance of soluble versus membrane-bound molecules established. Because T cell inhibition is associated, *in vitro* with a transient binding to - and the transmigration under MSC, we investigated which surface molecules could be involved in these processes.
Integrins such as LFA-1, Mac-1, and VLA-4 were identified on activated T cells. These could respectively interact with I-CAM-1-2, V-CAM-1 and CD90/Thy-1 expressed on MSC. MSC also expressed junctional adhesion molecule (JAM)-B and JAM-C which can bind VLA-4 and Mac-1 on T cells. Moreover both cell types expressed CD44, which could support T cell-MSC binding in the presence of hyaluronan (HA). Thus various pairs of receptor-ligand could be involved in T cell-MSC interactions. To identify which molecules were involved in cell binding, stimulated T cells were seeded over MSC in the presence or in the absence of blocking mAbs or excess of HA (known to inhibit CD44 interaction), and the number of bound T cells was assessed.
When T cells stimulated for 48 hours with mitogens were incubated over MSC for 1 hour at 37°C, only the anti-CD18 mAb (which inhibits both LFA-1 and Mac-1) significantly decreased cell binding (residual binding 83% of control, n=6 p<0.032

Wilcoxon). Combining mAb together did not increase cell binding inhibition. The contribution of CD44 could not be assessed because the addition of HA to the assay did not generate reproducible results. None of the mAbs, nor HA (from 1 ug to 1 mg/ml) affected T cell proliferation in the absence of MSC, or impaired MSC-induced T cell inhibition, thus indicating that integrin-induced cell-cell interaction was not the effector of the inhibition. Moreover experiments performed with transwell systems showed that T cell transmigration under MSC was not associated with inhibition as long as the compartment where the cells landed after transmigration was voluminous.
In conclusion, the binding of activated T cells to MSC is only partially mediated by beta-2 intergrins and seems not associated with MSC regulatory function. However cell-cell interactions may favor the action of soluble inhibitory effectors synthesized by MSC.

**P602**
**Pilot study on the measurement of calcineurin phosphatase activity on day 21 in allogeneic stem cell recipients**
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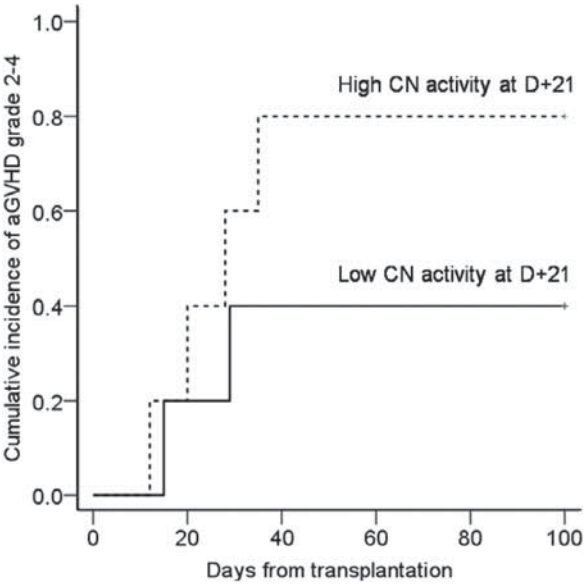
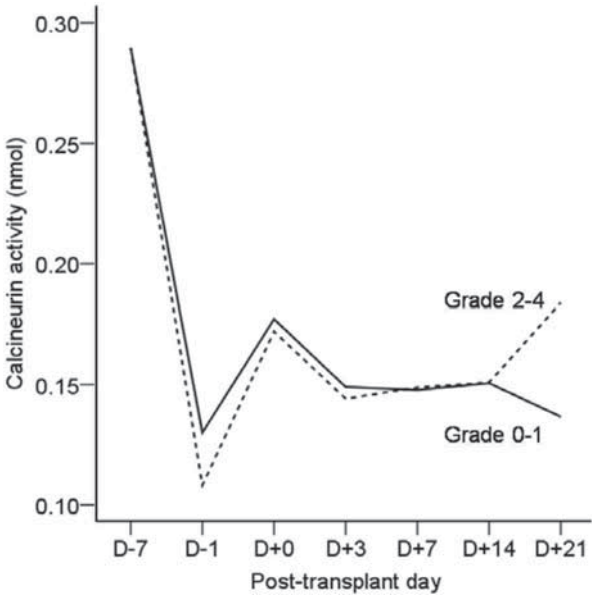
Background: Tacrolimus (TAC) suppress T-cell activation by inhibiting calcineurin (CN). CN activity was assessed in the allogeneic recipients who were treated with TAC for graft-versus-host disease (GVHD) prophylaxis to investigate whether CN activity was increased in patients with severe acute GVHD.
Methods: CN activity was analyzed in 10 consecutive patients who underwent allogeneic stem cell transplantation (SCT). TAC was administered at a dose of 0.03 mg/kg intravenously from day-1 to +21. TAC levels and CN activity was assessed on day-1 before TAC administration and days 0, +3, +7, +14, and +21. Target TAC concentration (15-20 ng/ml) was maintained during the current study.
Results: The cumulative incidence of acute GVHD (74.1% *vs.* 60.3%, p=0.888) and severe chronic GVHD (22.5% *vs.* 33.3%, p=0.539) were not different between high and low TAC trough levels. CN activity on day-1 was 0.12±0.09 nmol and had decreased from baseline level (0.29±0.15 nmol, p<0.001). There was no correlation between CN activity and TAC concentrations (r2=0.024). CN activity was steady-state during post-transplant day+0 to +14 regardless of acute GVHD, CN activity on day+21 for those with grade 2-4 acute GVHD showed higher CN activity (0.18±0.04 nmol) compared to those without grade 2-4 acute GVHD (0.14±0.05 nmol, p=0.462). Cumulative incidence of acute GVHD (40% *vs.* 80%, p=0.248) and chronic GVHD (20% *vs.* 70%, p=0.464) between low and high CN activity group were not significantly different.
Conclusion: Maintaining target TAC trough level did not correlate with the development of GVHD in allogeneic recipients. Although GVHD was higher for the high CN activity on day+21, this pilot study failed to demonstrate significant difference due to small sample size. However, the patients manifesting GVHD with high CN activity on post-transplant D+21 may need to be treated with other kinds of immunosuppressive agent regardless of drug level.

**[P602]**




**P603**
**Adaptation of a murine chronic GvH model to study graft-versus-myeloma effect after allogeneic transplantation**
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To elucidate the mechanisms behind graft versus tumor effect (GVT) and graft versus host disease (GVH), our laboratory adapted a murine model of allogeneic bone marrow (BM) transplantation using B10.D2 donor mice and Balb/cJ recipient mice that were inoculated with MOPC-315.BM myeloma cells. Balb/cJ recipient mice were intravenously (IV) injected with $2{,}5x10^5$ luciferase transfected MOPC-315.BM cells. At day 30 after inoculation, 6 mice received an autologous transplantation

(Balb/cJ cells) and 8 mice received an allogeneic transplantation (B10.D2 cells) by IV injection of $10x10^6$ BM cells and $70x10^6$ spleen cells. Prior to transplantation, both groups of mice were irradiated with 6 Gy. Tumor development was followed by measuring their bio-luminescence using VIVOVISION IVIS 200 (Xenogen). Immune responses were followed by taking blood samples before transplantation (day -2), and at days 7 and 19 after transplantation, analysing lymphocyte counts and NK, NKT and T-cell subpopulations. When mice showed signs of paraplegia or signs of GVH disease, they were sacrified and analysed for immune activation and regulation in different organs (blood, spleen, lymph nodes, thymus and bone marrow). *In vivo* imaging showed disappearance of the luciferase signal in 7 of the 8 allografted mice. All the mice that received an autologous transplantation developed myeloma disease. The recovery of myeloma diseased mice by this allogeneic transplantation could be attributed to an immune graft versus myeloma effect. Further analysis of the cellular subpopulations at sacrifice (Allografted *vs* Autografted mice) showed a decrease in regulatory T cells [% of CD4: Blood 5,5±2,6 *vs* 18,4±4,4 ($p=1,6x10^{-5}$); Spleen 20,6±8 *vs* 24,7±4,7 (p=0,29); BM 10,9±6.5 *vs* 48,1±6,4 ($p=1,79x10^{-7}$)] and activation (CD69) of both CD4 T lymphocytes [% of CD4: Blood 45,4±14,5 *vs* 14,6±8,1 (p=0,0006); Spleen 44,9±8,9 *vs* 23,1±5,5 (p=0,0002); BM 60,4±14,4 *vs* 46,2±5,6 (p=0,04)] and CD8 T lymphocytes [% of CD8: Blood 51,6±14,8 *vs* 12,3±6,7 ($p=6,2x10^{-5}$); Spleen 46±7,9 *vs* 11,2±2,7 ($p=2,5x10^{-7}$); BM 69,6±9,6 *vs* 37,6±10,9 ($p=8,1x10^{-5}$)]. The same trends were observed in simply allografted control mice.
This model will be used for studying the mechanisms behind graft versus tumour effect (antigen mismatches, activation of T cell subpopulations) and the effects of immune suppressors (e.g. rapamycin) on the graft versus tumour effect.

**P604**
**Intrinsic survival capacity of cord blood T-cells is heterogeneous and can be promoted by repeated low doses of recombinant interleukin-7**
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Background: Umbilical cord blood (CB) transplantation is known to be associated with delayed and defective immune reconstitution, in part because recent thymic emigrants (RTEs), have limited intrinsic survival. Interleukin-7 (IL-7) has been reported to increase the initial recovery of the graft-derived T-cells compartment. The aim of this study was to investigate CB-derived T-cell survival and to define a minimal effective dose of recombinant human IL-7 (rIL-7), considering that in addition to its anti-apoptotic effect, IL-7 may enhance immune reactions that occur in the host, including acute GVHD.
Methods: Fifteen CB were obtained after normal-term delivery, using the same procedure as for CB banking. T cells were isolated within 12 hours by negative magnetic bead sorting and cultured for 2 weeks either directly or after being frozen. Unstimulated T cell cultures were conducted in parallel in medium (RPMI supplemented with 10% normal AB serum) alone or supplemented with a range of rIL-7concentrations added either only once at day 0 (100 to 1000 pg/mL) or every day (10 to 100 pg/mL). Cell viability was assessed by flow cytometric scatter analysis and staining with 3,3'-dihexyloxacarbocyanine iodide [DiOC6(3)] and propidium iodide.
Results: In basic culture conditions, the majority of T cells had died over 2 weeks. There was heterogeneity in cell survival, with 2% to 50% viables T cells (median 15%) at day 6 of culture. Interestingly, the same intrinsic characteristics of survival were observed in T cells that underwent beforehand a freezing-thawing procedure. In cultures supplemented with rIL-7, we confirmed the efficacy of rIL-7 in maintaining CB T cell survival. A minimal dose of 25 pg/mL added daily was sufficient to induce the prolonged survival of CB T cells, with more than 95% of viable cells at day 6. Noteworthy, these concentrations allowed prolonged survival of CD T cells without inducing any cell proliferation, as shown by the absence of CFSE dilution. Similar results were observed comparing CB cells that had been frozen or not.
Conclusion: Repeated low-doses of rIL-7 are required to preserve the survival capacity of CB T cells without inducing their proliferation. These results could represent a framework for clinical studies aiming at improving T cell reconstitution following allogeneic CB transplantation. Repeated administration of low-doses of rIL-7 might be especially useful in patients with low serum levels of IL-7 after conditioning.

**P605**
**Intestinal toxicity after busulfan and cyclophosphamide treatment in piglets**
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Objectives: Chemotherapy treatment related toxicities are common among children undergoing Haemopoietic Stem Cell Transplantation (HSCT). Gastrointestinal complications, such as oral and intestinal mucositis, are related to increased risk of severe infections and increased mortality. The objective of this study was to investigate intestinal structural, functional and inflammatory markers after treatment with busulfan (Bu) and cyclophosphamide (Cy) in a piglet model of chemotherapy-induced mucositis.
Methods: Piglets were allocated to receive either 4 d of Bu plus 2 days of Cy (1.6 and 60 mg/kg/d, respectively, Bu-Cy group, n=9) or saline (controls, n=8) intravenously. After 6 days chemotherapy or saline, pigs were kept for another 5 days until when intestinal samples were collected for histomorphometry (villus height and crypt depth), activity of six brush border enzymes (disaccharidases, peptidases) and tissue levels of cytokines (IL-8, IL-1beta and TNF-alfa) in the proximal and distal intestine.
Results: Bu-Cy pigs had 40% shorter villi in the proximal intestine, relative to controls ($p<0.01$) while no difference was seen in the distal intestine. Crypt depths were smaller in both proximal and distal intestine (18-24%, $p<0.05$). Likewise, the activity of the brush border enzymes in the proximal intestine (sucrase, ApA, ApN and DPPIV) were 30-50% lower in Bu-Cy pigs compared with controls pigs ($p<0.01$ for all) while in the distal intestine only sucrase was lower in Bu-Cy pigs ($p<0.05$). No differences were found for maltase and lactase. IL-8 were 40% higher ($p<0.05$) in the proximal intestine while TNF-alfa levels were 50% higher in the distal intestine of Bu-Cy pigs ($p<0.05$). No difference was found for IL-1-beta.
Conclusion: The Bu-Cy treatment resulted in structural damage and reduced functions, especially in the proximal part of the small intestine within 5 days after the 6 days treatment protocol. A faster enterocyte turnover in the proximal versus the distal intestine may explain that chemotherapy induces more marked effects proximally. The associated increases in tissue IL-8 and TNF-alfa levels reflect local bacteria-stimulated inflammatory reactions. These may accelerate damage and prime the tissue to later development of graft versus host diseas following infusion of a transplantat. Pharmacological and dietary factors should be investigated as means to reduce the immediate intestinal effects of Bu-Cy treatment.

**P606**
**Fludarabine-cytabarine-melphalan combination – a novel high-dose chemotherapy conditioning regime for autologous peripheral blood stem cell transplant in lymphoma**
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Background: Fludarabine-Cytabarine-Melphalan combination (FLAM) is a novel combination chemotherapy which theoretically has the advantage of being able to provide Central

Nervous System penetration (high dose cytarabine) as well as using 2 non-cross resistant chemotherapy drugs (Fludarabine and Melphalan) that is lympholytic and which most of the patients on lymphoma treatment would not have prior exposure.
Methods: Selected patients with prior autologous peripheral blood stem cells collected(chemotherapy - GCSF mobilisation) were treated in Hospital Ampang from June 2011 till October 2011 with IV Fludarabine 30 mg/m$^2$ for 5 days, IV Cytarabine 2000 mg/m$^2$ over 2 hours for 5 days, IV Melphalan 140 mg/m$^2$ for 1 day. Autologous stem cells were reinfused subsequently. Most of the patient received GCSF from D3 post reinfusion until neutrophil engraftment. Patients were monitored in the ward until stable engraftment. Demographic data, disease and remission status and chemotherapy toxicity, engraftment details were collected during the admission.
Results: There were a total of 22 patients treated in total. 31.8% of the patients had Hodgkin Lymphoma, 68.1% had Non-Hodgkin's Lymphoma in which 50% was B cell lymphoma and 18.1% was T cell lymphoma. The main diagnosis was DLBCL (40.9%). 54.5% had no significant oral mucositis. There was only 1 (4.5%) patient with grade 3 oral mucositis, 6 (27.3%) with grade 1 mucositis and 2 (9.1%) with grade 2 mucositis . Median autologous peripheral blood stem cell dose was 4.7x10$^6$ CD34/kg. Median day of neutrophil engraftment was on Day +10 and median day of platelet engraftment was on Day +12. No grade 3 or grade 4 renal or liver toxicity was noted. There was 1 treatment related mortality (TRM) (4.5%) due to sepsis.
Conclusions: FLAM is a promising and feasible novel high dose chemotherapy regime that could be used in combination with autologous stem cell rescue in lymphoma. TRM is acceptable and engraftment rates were comparable with other regimes. It appeared to be quite tolerable and there were minimal chemotherapy related toxicities. Long term remission and complications as well as effectiveness of the regime remained to be evaluated.

**[P606]**

| Disease Remission Status | |
|---|---|
| CR 1 | 7 |
| CR > 1 | 4 |
| PR | 4 |
| Relapsed/Refractory | 6 |
| Primary Refractory | 1 |

| | | | |
|---|---|---|---|
| **Age(Years)** | Median | | **40.5** |
| | Range | | **14.3 to 57.9** |
| | | | |
| **Gender** | Male | | **59.1%** |
| | Female | | **40.9%** |
| | | | |
| **Hodgkin lymphoma** | Nodular Screlosis | 3(13.6%) | |
| | Mixed cellularity | 1(4.5%) | |
| | Lymphocyte rich | 1(4.5%) | |
| | Not specified | 2(9.0%) | **7(31.8%)** |
| | | | |
| **Non-Hodgkin's lymphoma** | **B cell** | | **11(50%)** |
| | DLBCL | 9(40.9%) | |
| | Mantle Cell Lymphoma | 1(4.5%) | |
| | Follicular Lymphoma | 1(4.5%) | |
| | | | |
| | **T Cell** | | **4(18.2%)** |

**P607**

**Determinants of higher pre-apheresis CD34+ count in patients undergoing autologous haematopoietic stem cell mobilisation**

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Peripheral blood (PB) pre-apheresis CD34+ counts have been shown to predict the CD34+ yield in patients undergoing

autologous stem cell collection. Determining correlates of PB pre-apheresis CD34+ counts can identify patients at higher risk of mobilization failure.

Methods: A total of 851 consecutive autologous apheresis procedures performed at our institution between 01/05 and 12/09 were included. We randomly selected half (N=415) of this study population as a study sample (Table), and preserved the rest for validation studies. As expected, in the study population 96% of patients with PB pre-apheresis CD34+ counts of >40/μL collected >2x$10^6$ CD34+cells/Kg on the 1st day of apheresis. We sought to determine patient and disease characteristics that are associated with higher PB pre-apheresis CD34+ counts (>40/μL). These factors included patient age (quartiles), gender, weight (quartiles), diagnosis, disease status at transplant (remission *vs.* active disease), number of prior chemotherapy regimens (<2 *vs.* ≥2), complete blood count on day of collection:

**[P607]**

**Table**

| | N=413 | (%) | PB CD34+>40μ/L (%) |
|---|---|---|---|
| **Diagnosis** | | | |
| Multiple Myeloma | **232** | 56% | **33%** |
| Non-Hogkin's Lymphoma | **132** | 32% | **46%** |
| Hodgkin's Lymphoma | **49** | 12% | **55%** |
| **Gender** | | | |
| Female | **160** | 39% | **35%** |
| Male | **253** | 61% | **43%** |
| **Duration of mobilization (days)** | | | |
| ≤ median | **261** | 68% | **50%** |
| >median | **125** | 32% | **18%** |
| **Age, (years)** | | | |
| ≤ 50 | **124** | 30% | **51%** |
| 51-60 | **132** | 32% | **40%** |
| 61-70 | **122** | 30% | **34%** |
| >70 | **35** | 8% | **23%** |
| **Weight, Kg (quartiles)** | | | |
| ≤ 70 | **104** | 25% | **32%** |
| 71-85 | **113** | 27% | **39%** |
| 86-95 | **82** | 20% | **38%** |
| >95 | **114** | 28% | **50%** |
| **Disease status at mobilization** | | | |
| Active disease | **87** | 21% | **37%** |
| No Active disease | **320** | 79% | **52%** |
| **Prior Chemotherapy regimens** | | | |
| >2 | **146** | 35% | **42%** |
| ≤ 2 | **266** | 65% | **38%** |
| **Blood count pre- apheresis Hemoglobin (g/dL)** | | | |
| ≤ 10 | **85** | 21% | **40%** |
| >10 | **324** | 79% | **40%** |
| **WBC (x$10^9$/L)** | | | |
| <4 | **117** | 29% | **29%** |
| ≥4 | **292** | 71% | **44%** |
| **ANC (x$10^9$/L)** | | | |
| < median | **251** | 61% | **34%** |
| ≤ median | **158** | 39% | **49%** |
| **Platelets, (x$10^9$/L)** | | | |
| ≤ 150 | **75** | 18% | **33%** |
| >150 | **334** | 82% | **42%** |
| **WBC (x$10^9$/L) / ANC (x$10^9$/L)** | | | |
| WBC >4 / ANC> median | **157** | 38% | **50%** |
| WBC>4 / ANC ≤ median | **135** | 33% | **38%** |
| WBC≤ 4 / ANC > median | **116** | 28% | **29%** |

Totals may vary because of missing data

hemoglobin (≤10 *vs.*>10 g/dL), WBC (<4 *vs.*≥ 4 x$10^9$/L), ANC (<vs.≥ median), and platelet count (≤150 *vs.*<150 x$10^9$/L), and days to 1st apheresis procedure from start of mobilization regimen (≤ *vs.*> median).
Results: On univariate analysis, shorter duration from start of mobilization therapy to 1st day of collection (odds ratio [OR]=4.7, p<0.001), and weight >95 Kg (>4th quartile), (OR=1.8, p=0.01) were associated with a CD34+count >40/μL; whereas a diagnosis of multiple myeloma (OR=0.5, p=0.002), age >60 years (OR=0.6, p=0.009), pre-apheresis WBC <4 x $10^9$/L (OR=0.5, p=0.004) and ANC (<median p=0.003) were associated with PB CD34+ counts of ≤40/μL. These factors, with the exception of age remained significant on multivariate analysis. Shorter duration of mobilization (OR=8.1, p<0.001) had the strongest association with PB pre-apheresis CD34+ count >40/μL. Lower WBC and ANC counts were associated with lower PB CD34+ counts, but this effect was more pronounced for patients who had both low WBC and ANC (OR=0.4, p=0.001) than those who had only low ANC (OR=0.5, p=0.025). Weight >95 Kg was still associated with higher PB CD34+ count, (OR=1.9, p 0.01). There was no association between PB CD34+ count and any of the remaining factors evaluated.
Conclusion: Our data suggest that patients who take longer to mobilize might benefit from early intervention with novel mobilization strategies.

**P608**
**High-dose melphalan followed by two doses of bortezomib as conditioning regimen for the patients with multiple myeloma may increase response rate after autologous stem cell transplantation**
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Background: High dose melphalan with autologous hematopoietic stem cell transplantation(ASCT) is recommended as standard treatment for patients younger than 65 years with multiple myeloma. But complete remission (CR) rates are about 30% to 40% after ASCT and most of patients will relapse at a median time of 2 to 3 years. Bortezomib has been demonstrated synergistic effects with melphalan. *Ex-vitro* study showed that the bortezomib using after HD-melphalan could induce more appotosis. We performed a pilot study by using high-dose melphalan followed by two doses of bortezomib as conditioning regimen for ASCT in Chinese patients with MM to evaluate its safety and efficacy in improving response rate and survival.
Methods: Patients younger than 65 years with newly diagnosed MM were enrolled. The induction regimen had no limited. The conditioning regimen consisted of administration of melphalan 200 mg/$m^2$ on day -2 and bortezomib 1.0 mg/$m^2$ on days +4 and +1 after peripheral blood stem cell infusion. Melphalan was given 140 mg/$m^2$ in patients older than 55 years or patients with mild to moderate organ insufficiency. Maintenance therapy with thalidomide was given 2 to 3 months post-ASCT.
Results: Overall 35 patients with newly diagnosed MM were enrolled between November 2008 and November 2011. The median age of enrolled patient was 49 years (range, 34-65) years. Before ASCT patients' remission status included CR (n=14), near CR (nCR, n=9), very good partial remission (VGPR) (n=3), partial remission (PR, n=7), minimal remission (MR, n=1), relapse (n=1). Eighteen patients developed grade 1 to 2 gastrointestinal adverse events after conditioning. No grade 3 to 4 peripheral neuropathy (PN) was reported. There was no treatment-related death. Overall 93.1% patients achieved CR/nCR, including 18 patients with CR, 15 patients with nCR. Response rates of at least VGPR and CR/nCR were 100% and ≥ 92%, regardless of different induction therapy. With a median follow-up of 335 days (range, 45-10814) days after ASCT, 32 patients were alive, 8 patients relapsed with a median time of 315 days (range, 90-540) days. The estimated 2-year PFS was 53.7%±11.8% and the probability of 2-year OS was 85.5%±10.6%.
Conclusion: HD-melphalan followed by two doses of bortezomib as conditioning regimen may achieve promising response rates in CR/nCR after ASCT with low toxicities. Randomized studies and long-term follow-up are needed to assess its efficacy in improving survival.

## Early Complications

**P609**
**Genetic variations in T-cell activation and effector pathways modulate alloimmune reactivities and have prognostic significance following allogeneic haematopoietic stem cell transplantation**
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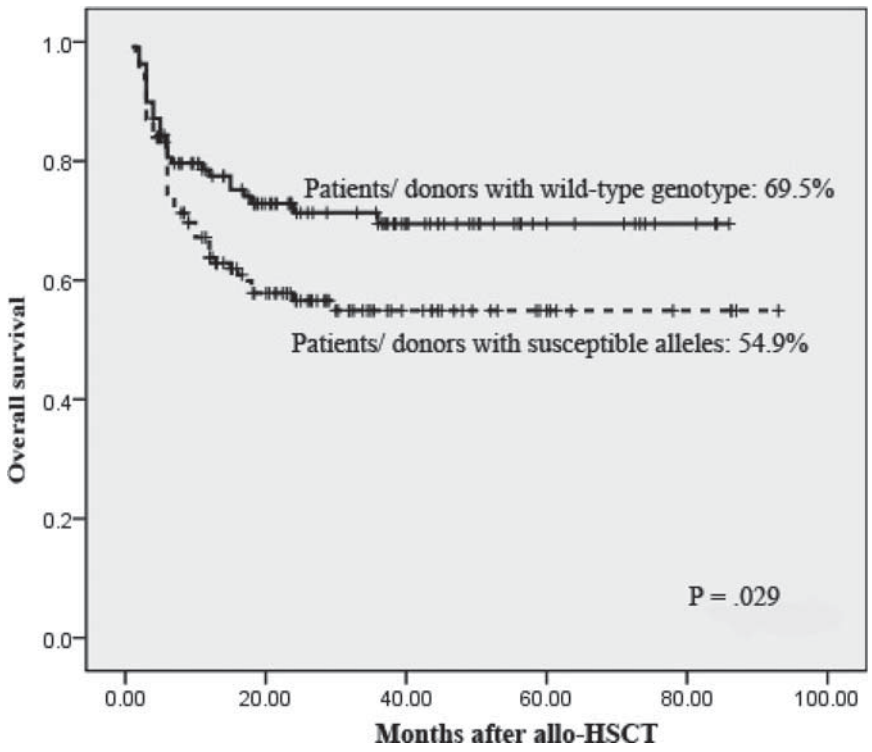
Objectives: CD28, inducible co-stimulator (ICOS) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) belong to the same family of T-cell costimulatory molecules. After activation, three effector pathways have been described for T-cell cytotoxicity: granzyme B/perforin, Fas/Fas ligand (FasL) and secreted molecules such as tumor necrosis factor-alpha. Near recently, several important polymorphisms have been identified and reported to be associated with the risk of autoimmune diseases, malignancies and allograft rejection in solid organ transplantation patients. However, such information is less available in allogeneic hematopoietic stem cell transplantation (allo-HSCT). In this study, we first investigated the influence of those polymorphic features on the abilities of T-cell alloimmune responses, including inducing GVHD, prevention of cytomegalovirus (CMV) infection and recurrence of the malignancy in allo-HSCT setting.
Methods: We analyzed 10 single nucleotide polymorphisms (SNPs) in CD28, ICOS, CTLA-4, Granzyme B, Fas and FasL genes in 2 independent cohorts including 138 pairs of recipients and their unrelated donors (URDs) and 102 pairs of recipients and their HLA-identical sibling donors.
Results: (1) We found unrelated donor with Granzyme B +55 mutated genotype (AA) was an independent risk factors for grades II-IV aGVHD (P =0.024, RR=1.811). While unrelated donor with CTLA-4 CT60 mutated genotype (AA) was protective (P =0.025, RR=3.806). (2) However, donors with CTLA-4 CT60 AA genotype were contributed to the development of early CMV infection (P<0.0001, RR=0.383) and relapse post-HSCT in AML patients (P=0.047, RR=2.792). Furthermore, AML patients with Fas -670 homogeneous mutated allele (TT genotype) also had a higher risk of relapse (P= 0.003, RR=3.823). (3) Patients were stratified into 2 groups including 131 patient-donor pairs were with at least one susceptible allele (patients receiving CTLA-4 CT60 AA donor, patients receiving GranzymeB +55 AA donor, AML patients with Fas -670 TT genotype or with all), and 109 patient-donor pairs were with wild-type genotypes. The presence of those susceptible alleles in donor and/ or recipient resulted in a reduced overall survival (Figure).
Conclusions: Our data highlight the important effect of genetic variations in T-cell activation and effector pathways from the donors and recipients on the outcomes of allo-HSCT, which suggests that genotype analysis of these genes can be used for pre-HSCT risk assessment.

[P609]




**P610**

**The impact of Toll-like receptor 4 polymorphisms on complications after allogeneic HSCT**

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Toll like receptor 4 (TLR4) is an important pathogen recognition receptor. Recent evidence suggests that the ability to respond properly to TLR ligands may be impaired by single nucleotide polymorphisms within TLR genes. HSCT is associated with several complications such as graft-versus-host disease (GVHD) and an increased risk of infections. Therefore polymorphisms on TLR4 may influence individual susceptibility to GVHD or infectious complications. The purpose of this study was to investigate the influence of TLR4 polymorphism on complications after allogeneic HSCT. A total of 264 patients undergoing allogeneic HSCT and their respective donors were retrospectively genotyped using pyrosequencing for two polymorphisms in TLR4 (Asp299Gly and Thr399Ile). Allele frequency of TLR4 was similar in the patients and donors group. The wild type AA and CC genotype was present in 89% of the patients. No homozygous mutated genotype was found for both polymorphisms. An increased relative risk of acute GVHD was observed in the group of patients with the AG genotype (RR 2.09 95% CI:1.17-3.37) although the difference was not significant (p=0.08) in univariate analysis. In addition, the incidence of fungal infections in patients bearing the AG genotype was significantly higher compared with the AA genotype (p=0.044). TLR4 Thr399Ile polymorphism was not associated with clinical complications or transplantation outcome. Other post transplant characteristics such as cytomegalovirus reactivation, chronic GVHD, overall survival and disease free survival were unrelated to the presence of these polymorphisms. Typing for the Asp299Gly polymorphism in TLR4 may result in a reduced incidence of fungal infections in patients undergoing allogeneic HSCT. A larger prospective study is required to define genetic susceptibility to complications after allogeneic HSCT.

**P611**

**N-terminal pro-brain natriuretic peptide role in early diagnosis of hepatic veno-occlusive disease and severe sepsis following allogeneic haematopoietic stem cell transplantation – a single-centre experience**

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Introduction: Hepatic veno-occlusive disease (VOD) and severe sepsis (SS) are major complications of allogeneic hematopoietic stem cell transplantation (allo-HSCT) whit significant impact on early transplant related mortality (TRM). In both conditions early specifically tailored therapy is mandatory therefore identification of serum markers with high sensitivity and specificity for diagnosis could have an impact on survival.
Objective: This retrospective analyze aimed at evaluating the importance of N-terminal pro-brain natriuretic peptide (BNP) assessment in early detection of VOD and SS. Method The study group consisted of 11 recipients of allo-HSCT with a mean age of 33.18±17.42 and a sex ratio of 4.5:1 (male:female). Indications for allo-HSCT included both malignant (10/11) and non-malignant (1/11) hemopathies. Busulfan based conditioning regimens were applied in 90.9% of case, without use of total body irradiation. All patients received primary prophylaxis for infections with cyprofloxacine, fluconazole, TMP/SMX, acyclovir and for VOD with unfractionated heparine (120UI/kg). The nursing of patients was performed in laminar airflow rooms. Diagnosis of VOD was established based on Baltimore criteria. Electrochemiluminescence sandwich immunoassay was used for measuring plasma BNP before (day -7, 0) and after (day +7, +14) allo-HSCT.
Results: VOD and SS were each diagnosed in 36.36% of patients. In patients diagnosed with VOD or not the mean values of

BNP evaluated on days -7/0/+7/+14 were 53/2270/6213/10330 pg/ml and 53/79/405/43 pg/ml (p=0.66/0.009/0.31/0.04) respectively, with relevant significance of BNP for VOD only on days 0 and +14. Comparative analyses of the group without complications and the one with SS revealed a statistically significant higher value of BNP only on day +7 (p=0.009). Median days of onset for VOD and SS were +15 (range 12-18), respectively +9 (range 7-13). Our findings showed an ascendant pattern of BNP values starting with day 0 with a peak level before onset of VOD.
Conclusion: Plasma BNP, although not routinely monitored in HSCT setting, seems to be a predictive indicator for onset of both VOD and SS. Strategies for early detection of VOD and SS in allo-HSCT should be established, more frequent measurements of plasma BNP possibly bringing valuable data for preemptive treatment of these complications.

**P612**

**Clinical significance of glutathione S-transferase M1 and T1 genes polymorphisms for outcome after allogeneic haematopoietic stem cell transplantation**

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Background: Despite significant progress in allogeneic hematopoietic stem cell transplantation (allo-HSCT), this procedure is still associated with substantial morbidity and mortality. The main complication after allo-HSCT which affects survival remains graft versus host disease (GvHD). The genetic polymorphisms in genes coding cytokines, chemokines and metabolic enzymes have been shown to influence GvHD, toxic complications and outcome after allo-HSCT. This study aimed to determine association between polymorphisms of glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) genotypes with the outcome of allo-HSCT performed between August 2003 and October 2011 in our center.
Materials and Methods: The allelic variants of GSTM1 and GSTT1 genes were determined in 109 patient/donor pairs by real-time polymerase chain reaction. Patients characteristics were: median age-36 (range 18-65), underlying diseases-hematologic malignancies-in 103 and aplastic anemia–in 6 cases. Donors were HLA-identical sibling-in 70 and unrelated-in 39 transplants, the median age-37 (range 14-65). The studied end points were GvHD, haemorrhagic cystitis, toxicity and venooclusive disease (VOD) of the liver and survival. The Spearman's rank correlation test and the Kaplan-Meier method were used in statistical analysis.
Results: The allelic distribution of examined polymorhisms was similar to that reported in Caucasoid population. Acute-GvHD was recognized in 42/109 (38,5%) and chronic - in 51/109 (46,8%) of patients. Overall survival in analysed group was 66,1%. Our study revealed significant correlation between the occurrence of aGvHD and the GSTM1-positive/GSTT1 - null genotype in recipients (P=0.0167). Besides we found a significant correlation between the GSTT1 - positive genotype in donors and increased treatment related mortality at the 180 day in patients (P=0.029). We also revealed some relations indicative of the trend between increased risk of mortality and GSTM1-positive/GSTT1-positive genotype (P=0.077) and GSTM1-positive/GSTT1-null genotype (P=0.082) in recipients. We couldn't find any significant correlations between examined polymorphisms and the rest of analysed end points (P=ns).
Conclusion: Our data revealed that GSTM1-positive genotype is correlated with aGvHD and increased risk of mortality after allo-HSCT. These findings confirmed some previously published data but they should be studied prospectively and in the larger group of patients.

**P613**

**Evaluation of transjugular liver biopsy in the diagnosis of early hepatic dysfunction after allogeneic haematopoietic cell transplantation**

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Introduction: Liver biopsy might be necessary in the diagnosis of liver dysfunction after allogeneic stem cell transplantation (allo-SCT). Due to the complications related to transparietal (TP) access, transjugular liver biopsies (TJLB) are usually preferred, specially in the early period. We retrospectively analyzed the role of TJLB in the diagnosis of early hepatic dysfunction after allo-SCT.
Methods: All consecutive allo-SCT recipients undergoing TJLB in our centre from May 1997 to September 2011 were included. Median follow-up for survivors was 28 months (1-57). According to our protocol, TJLB instead of TP access was preferred in patients with platelet count <50x10e9/L, coagulation abnormalities or unstable medical condition. TJLB were performed using a plastic needle guide through jugular access. Pathological samples were analyzed by an experienced pathologist in the centre and revised retrospectively.
Results: A total of 23 TJLB were performed on 20 patients (13% of the 153 allo-SCT performed during the study period). Median age was 29 years (range 17-64). Most patients received myeloablative allo-SCT mainly for AML. Most frequent clinical suspicions before TJLB were venooclusive disease (VOD) (n=10, 44%) and GVHD (n=10, 44%). Transjugular access was chosen because of thrombocytopenia (n=11, 47%), coagulation abnormalities (n=2, 4%) or both (n=10, 43%). Median bilirrubin level at the time of TJLB was 8 mg/dL (1-45). Median time from transplantation to TJLB was 38 days (range 12-152). The most common histological findings were iron overload (n=10, 43%), cholestasis (n=8, 35%), and portal fibrosis (n=7, 30%). A definitive histopathological diagnosis was obtained from 15 out of 23 biopsies (65%), including 5 (21%) VOD, 5 toxic hepatitis (21%), 2 liver GVHD (8%), 2 cholangitis lenta due to sepsis (8%) and 1 CMV hepatitis (4%). Eight (35%) biopsies were non-diagnostic, 6 due to unspecific findings/mild iron overload and 2 due to insufficient sample size. Main clinical suspicion was confirmed after the biopsy in 8 (35%) cases while in 9 (39%) it revealed another diagnosis which led to a new therapeutic approach. Most frequent TJLB-related complications were subcutaneous hematoma (n=3, 13%). There was 1 (4%) TJLB-related death because of severe bleeding.
Conclusion: TJLB appears to be relatively safe in the early post-allo-SCT period although its usefulness as a diagnostic tool might be impaired due to unspecific findings or small sample size.

**P614**

**The role of C-reactive protein and procalcitonin levels in the differential diagnosis between infection and engrafment in peri-engraftment period after haematopoietic stem cell transplantation**

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The purpose of this study was to investigate the association of serum levels of C-reactive protein (CRP) and procalcitonin (PCT) in infection and engraftment in periengraftment period after hematopoietic stem cell transplantation (HSCT).
Seum CRP and PCT levels were measured in 32 patients who had undergone HSCT.
Serum CRP level was increased in 21 (65.6%) patients, PCT level was increased in 5 (15.6%) patient in periengraftment period. There is no any relationship with increased CRP levels

and infection or engraftment syndrome (p>0,05). The increased PCT level related to infection (p<0,05). The increased CRP level with normal PCT level related to engraftment, both of high level of CRP, PCT related to infection (p<0,05).
Our results support that while high levels both of CRP and PCT associated with infection, only high level of CRP (PCT hight not accompanied) related with engraftment in periengraftment period.

Table

| Characteristics of patients and transplantations | Number |
|---|---|
| Total patients | 32 |
| Mean age (year) | 7.46 ± 4.65 |
| Male / Female | 16/16 |
| Diagnosis | |
| Malign disease | 13 |
| Non-malign disease | 19 |
| Ttpe of transplantation | |
| Autologous | 4 |
| Allogeneic | 28 |
| Source of stem cell | |
| Bone marrow | 27 |
| Peripheral blood | 4 |
| Bone marrow + peripheral blood | 1 |
| Conditioning regimen | |
| Myeloablative | 29 |
| Non-myeloablative | 3 |

**P615**
**The role of cardiac biomarkers after allogeneic haematopoietic stem cell transplantation: can we predict cardiac dysfunction?**
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Background: Cardiac complications are frequently found life-threatening conditions after hematopoietic stem cell transplantation (HSCT). Serial measurements of cardiospecific biomarkers can be a valid diagnostic tool for early identification, assessment, and monitoring of cardiotoxicity. The aim of the study was the assessment of the cardiotoxicity using cardiac biomarkers - N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high sensitive cardiac troponin T (hs-cTnT) during HSCT.
Methods: Thirty-seven patients who were treated with allogeneic HSCT for hematologic diseases at median age of 28 years (range: 19-58) at time of HSCT were studied. Conditioning regimen included either chemotherapy without total body irradiation (TBI) or combination of chemotherapy with TBI. Twenty-nine (78,3%) patients were treated with prior ANT therapy (with median cumulative dose 250 mg/m$^2$, range: 100-470). Together 148 blood samples were evaluated. Cardiac biomarkers were measured serially before conditioning regimen and at days 1, 14 and 30 after HSCT. In all patients, echocardiograms were performed before conditioning regimen and 1 month after HSCT.
Results: Eleven patients (29,8%) had persistently increased NT-proBNP, 14 patients (37,8%) had only transient elevations and 12 patients (32,4%) had no elevations [median (interquartile range) values at 14 days, 2023 (1623-2914) pg/ml *vs* 653 (470-836) pg/ml *vs* 114 (60,8-211) pg/ml, P<0,01]. Only patients with persistently increased NT-proBNP had significant elevations in hs-cTnT concentrations at day 14 (P<0,01) and a significant worsening of the left ventricular diastolic and systolic parameters were found after 1 month. Five of 11 (45,4%) patients with persistently increased NT-proBNP developed clinical manifestation of cardiotoxicity.
Conclusions: Persistently increased NT-proBNP for a period exceeding 14 days after HSCT is strongly associated with development of echocardiographic abnormalities and might be used for identification of patients at risk of developing cardiotoxicity and requiring further cardiological follow up.
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**P616**
**Prognostic significance of plasma vascular endothelial growth factor in patients undergoing allogeneic stem cell transplantation**
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The aim of this study was to evaluate the prognostic value of vascular endothelial growth factor (VEGF) in patients undergoing allogeneic stem cell transplantation (alloSCT). We analyzed relationship between VEGF levels, risk and severity of veno-occlusive disease (VOD), early relapse and impact on engraftment. Study group comprised 32 patients with acute myeloid leukemia (16), chronic myeloid leukemia (7), acute lymphoblastic leukemia (3) and other hematologic malignancies (7). Mean age was 41 years (range 16-60). 23 were grafted from unrelated and 9 from related donor. Majority of patients received reduced intensity conditioning. VEGF was measured by ELISA before conditioning, on day 0, upon engraftment and in case of VOD. Moderate to severe VOD with capillary leak syndrome developed in 15% of patients. VEGF levels were not different in patients who developed VOD compared to other alloSCT patients (p>0.05) and control group (p>0.05). Higher levels of VEGF were observed in patients with early relapse after alloSCT (p=0.03) and were associated with delayed engraftment (p<0.01). Elevation of VEGF after conditioning, on day 0, was of highest prognostic significance for disease progression during 100 days after alloSCT.
Our preliminary data indicates that high level of VEGF may be considered as unfavorable factor for engraftment and early relapse after alloSCT in patients with hematologic malignancies. Development of VOD was not associated with alteration of VEGF concentrations.

**P617**
**Calcitriol receptor gene polymorphisms and clinical outcomes of HLA-matched sibling haematopoietic stem cell transplantation**
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Objectives: The immunoregulatory action of calcitriol, which is also known as the vitamin D has been investigated recently, and polymorphisms of the calcitriol receptor gene have been known to be associated with infection and malignancy. We hypothesized that polymorphisms of this gene might affect clinical outcomes of allogeneic hematopoietic stem cell transplantation (HSCT).
Methods: Three calcitriol receptor gene polymorphisms (BsmI G>A: rs1544410, ApaI G>T: rs7975232 and TaqI T>C: rs731236) were genotyped in 152 patients who underwent HLA-matched

sibling allogeneic HSCT in a single institution between 1998 and 2005. Frequencies of infection, graft-versus-host disease (GVHD), overall survival (OS) and disease-free survival (DFS) were compared according to genotypes and haplotypes.
Results: For ApaI, infection was more frequent in patients with the non-TT genotypes (GG or GT) than those with the TT genotype (87.3% *vs.* 61.6%; p=0.061). Grade II–IV acute GVHD developed less frequently in patients with the ApaI TT genotype than those with the non-TT genotypes (7.7% *vs* 35.3%; p=0.059). For TaqI and BsmI genotypes, there were no statistical differences in frequency of infection and acute GVHD (p=0.84 and p=0.30, respectively). However, no calcitriol receptor genotype of recipients showed association with development of chronic GVHD. In the ApaI-TaqI haplotype analysis, patients with TC haplotype had significantly longer OS and DFS than those without TC haplotype in univariate analysis (p=0.022 and p=0.038, respectively). In multivariable analysis, ApaI-TaqI haplotype as well as ApaI and TaqI genotype were independent prognostic factors of OS (HR=3.03, 95% CI: 1.320–6.937; p=0.009 for ApaI-TaqI haplotype and TaqI genotype, and HR=3.03, 95% CI: 1.084-8.403; p=0.035 for ApaI genotype), and TaqI genotype and the ApaI-TaqI haplotype were also independent prognostic factors of DFS (HR=5.24, 95% CI: 1.255–21.849; p=0.023 for both).
Conclusion: Our results showed that ApaI genotype might be associated with acute GVHD and infection, and TaqI genotype and ApaI-TaqI haplotype were independent prognostic factors in terms of OS and DFS.
This study suggests that the genotype and haplotype of calcitriol receptor in recipient might be associated with clinical outcome of sibling HLA-matched HSCT.

**P618**

**Plasma zinc, copper and selenium in subjects planned for haematopoietic stem cell transplant and their change during treatment**

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Background: Zinc (Zn), copper (Cu) and selenium (Se) are essential nutrients for haematopoiesis, immunocompetence, antioxidant defence, DNA structure and replication. Their status maybe altered in SCT recipients due to increased requirements, impaired intake or losses secondary to disease or treatment side effects.
Monitoring of Zn, Cu and Se in patients receiving artificial nutrition support (ANS) is recommended however the systemic inflammatory response (SIR) alters levels irrespective of intake. Plasma Zn and Se fall due to increased capillary permeability and tissue redistribution whereas plasma Cu rises with elevated caeruloplasmin synthesis. This observational study aimed to consider SIR and identify subjects at risk of deficiency during SCT.
Methods: Plasma Zn, Cu and Se were measured prospectively from pre-assessment to discharge on days -1, +6, +20, +34, +48 and +62 of SCT. CRP was simultaneously measured to indicate a SIR. Data regarding ANS was recorded.
Results: 60 subjects were analysed according to transplant type; autologous (auto n=19), reduced intensity conditioned (RIC n=23) myeloablative conditioned allogeneic (MA n=18). Plasma Zn and Se were negatively correlated with CRP whereas plasma Cu showed positive association therefore values recorded when CRP >20 mg/L were excluded to avoid confounding by SIR.
Pre/post-SCT plasma Zn was within range for auto and RIC groups however medians for MA SCT were 9.3 µmol/L (n=13, IQR 8.2–10.7) pre and 10.0 µmol/L (n=18 IQR 8.9–11.1) post SCT. Overall, Zn was below the reference range in 28% (n=11) prior to and 19% (n=6) post SCT.
Adjusted Cu values were within range pre and post SCT. Median Se was normal pre-SCT but reduced post-SCT in auto (p=0.006) and MA (p=0.003) subjects. Levels suggested deficiency in the auto group post SCT; median Se 0.73 (n=11, IQR 0.70–0.89). Overall the proportion with Se < reference range increased from 9% (n=4) pre-compared to 20% (n=12) post SCT (p=0.004).
ANS use of > 4 days by group was 5% (n=1) autos, 52% (n=12) RIC and 89% (n=16) MA (p=0.000).
Discussion: Plasma Zn was low pre MA SCT and remained borderline deficient despite ANS use in 89% of subjects. Increased Zn intake may benefit this group, but inappropriate supplementation causes iatrogenic Cu deficiency hence a targeted approach is needed. Plasma Se significantly reduced in MA and auto SCT. Final levels approached deficiency in auto recipients who also received the least ANS suggesting oral Se intakes maybe inadequate.

**P619**

**Phagocytic activity in monocytes subpopulations after haematopoietic stem cell transplantation in paediatric patients and young adults**

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Background: Phagocytosis of monocytes presents a major mechanism contributing to the clearance of pathogens and cell debris. Immune reconstitution after hematopoietic stem cell transplantation (HSCT) is associated with a compromised innate and adaptive immune response. Phagocytic activity in monocyte subpopulations may be similarly altered during the post-transplant period and during adverse events after HSCT. We analyzed phagocytic activity of three monocytes subpopulations before, during and up to one year after HSCT, as well as during transplant-related adverse events.
Patients and Methods: 25 children and young adults with hemato-oncological malignancies and immunodeficiency disorders (median age of 11.0 years) were enrolled after informed written consent. FITC-labeled Escherichia coli bacteria were used to assess phagocytic activity of monocyte subpopulations CD14++/CD16- (M1), CD14++/CD16+ (M2) and CD14+/CD16++ (M3) from a control group of healthy children (n=36) and the patient group. by flow cytometry (Figure 1).
Results: Median data acquisition period of phagocytic activity in monocyte subpopulations for the patient group was 213 days (range 45-382 days). By analysis of covariance (ANCOVA) phagocytic activity was not influenced in any of the three monocyte subpopulations by age or sex in the healthy control group. During the post transplant period, a significant increase of phagocytic activity of M2 CD14++/CD16+ monocytes was observed on day 30 after HSCT ($P < 0.001$). Phagocytosis was significantly reduced in M3 CD14+/CD16++ monocytes during sepsis or bacteremia ($P < 0.01$). During veno-occlusive disease (VOD), a significant increase of M2 CD14++/CD16+ monocytes ($P=0.001$) and a significant decrease of phagocytic activity in M1 CD14++/CD16- monocytes ($P < 0.001$) was observed. During VOD therapy, M2 CD14++/CD16+ monocytes ($P < 0.01$) decreased significantly and reached baseline levels. Phagocytic activity was not altered in any of the three monocyte subpopulations during GvHD and during treatment of acute GvHD.
Conclusion: Different patterns of phagocytic activity of monocytes subpopulations were observed during adverse events after HSCT. We conclude that these alterations contribute to the altered immune response accompanying the adverse events after HSCT.

[P619]

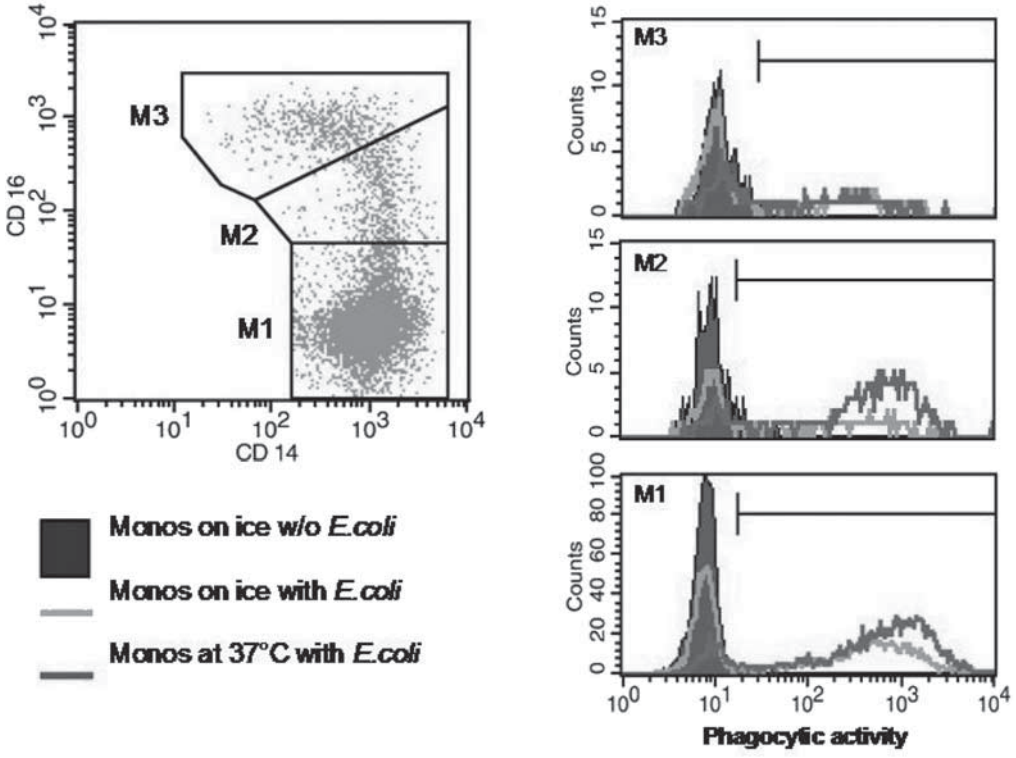


**Figure 1. Phagocytic activity of monocyte subpopulations CD14++/CD16- (M1), CD14++/CD16+ (M2) and CD14+/CD16++ (M3) was assessed by FITC-labeled *E. coli* via flowcytometric analysis.**

**P620**

**Audit of nutritional careof patients undergoing autograft**

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The nutritional status of a patient adversely affects outcome. ITU's routinely start feeding protocols on patients at admission to improve outcomes. Many patients prior to starting high dose chemotherapy have suffered significant toxicities and are malnourished at the beginning of the transplant process. Conditioning therapy results in mucositis and patients are often unable to tolerate enteral feeding. This is exacerbated by the patient's psychological state with many being depressed or showing features of food avoidance and lack of appetite related to experience of nausea post chemotherapy. Most patients are managed using oral nutritional support. Studies looking at the use of nasogastric, nasojejunal feeding or intravenous feeding have not outlined best practice as each is associated with both risks and problems of acceptance. In Leicester patients are all assessed prior to HPCT admission and oral supplementation with high calorie drinks is standard practice.

The aim of this audit was to assess the effectiveness of our current nutritional management strategy for patients undergoing autologous HPCT for myeloma and lymphoma and to try to identify areas for improvement.

Method: Data was collected retrospectively for all patients treated between Nov 2008 and Sept 2011 using medical notes and dietetic notes. Patients' weight (BW) on Day 0 through to Day +100 was identified along with any alternative feeding routes used.

Results: 109 patients were transplanted, Males 71, female 38. Median age was 56.3 (19-71years), Conditioning used was Melphalan for MM or BEAM for lymphoma. On admission median BMI was 27.1 kg/m$^2$ (16.54-49.87). Information was available for only 63 patients on discharge. The average weight lost per patient during transplantation was 3.06 kg (range 14.3 loss to gain 3.5 kg), 24 patients (38% of total) lost >5% BW and 6 patients (9.5%) lost more than 10% BW during transplantation. By 100 days post transplant average weight loss had increased to 3.97kg (4.71% starting BW) (range 16.9 loss to gain 3.6 kg) with 41.4% of patients loosing >5% BW. 10/63 (15.9%) patients had received an alternative feeding regimen (NG feeding 6, PEG/PEG-J 3, PN 1).

Conclusion: The audit identifies significant weight loss in many patients which is on-going at Day 100 depite resolution of mucositis. The use of alternative feeding methods to provide adequate nutritional support including post-discharge needs must be explored to improve patient recovery time and reduce morbidity.

**P621**

**Use of PEG- feeding in Allo-HPCT**

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The outcome of patients undergoing intensive chemo-radiation therapy associated with HPCT is adversely affected by the nutritional status of the patient. Pretransplant many patients have already undergone chemotherapy associated with severe

nausea and mucositis and may be nutritionally stressed at the outset. Combining the pre transplant state with the effect of mucositis during transplantation and the issue of gut GVHD is a potent cause of morbidity and mortality in this patient group. Many units use parental nutrition in this population but this is not without risk and does not aid gut health and performance. We have previously reported on our experience of the use of elective PEG-J insertion in this population group. The data presented here is an expansion of this previous work in a larger cohort of patients.
Method: All patients undergoing allo-HPCT are assessed pre transplant by a nutritional team. PEG-J insertion is carried out 2-4 weeks prior to transplantation. Patients who are malnourished at referral are commenced on nighttime PEG-J feeding prior to admission. All other patients are commenced at the time of admission and continued until discharge or post discharge when a preset target weight has been reached and maintained.
Results: 64 patients were transplanted between Nov 2008 and Sept 2011 54 of whom are evaluable. Male 37, Female 27, Mean Age 48.6 (range 20-73 years). Indications for transplantation are 20AML, 5ALL, 12NHL, 5HD, 4CLL , 1CML 1MM, 1AA. Donor sourse matched sibling or MUD. At admission the average BMI was 26.1 (16.4-41.1). 38 patients had a PEG-J inserted. Average length of PEG-J feeding was 75 days (0-651). Average weight loss at discharge was 1.95 kg (9.9 loss to 4.75 kg gain) and 3.73 kg (19.8 loss to 1.8 kg gain) for PEG-J and non-PEG-J patients respectively. At 100 days post HPCT average weight loss was 0.98 (9 loss to 9.9 kg gain) versus 5.02 kg (21.8 loss to 5.15 kg gain) in PEG-J and non-PEG-J feed patients. 28% of non-PEG-J managed patients required PN versus only 15% in the PEG-J group. 18.5% patients had an infection at the insertion site requiring antibiotics. Tube removal occurred 227 (35-658) days after removal.
Conclusion: PEG-J feeding can be safely undertaken in patients undergoing Allo-HPCT. PEG-J feeding reduces weight loss and minimises PN use and aids in patients recovery.

**P622**
**The relationship between nutritional status of lymphoma patients and the course after autologous stem cell transplantation**
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Background: There is an unmet need for developing the reliable and useful screening methods evaluating nutritional risk in lymphoma patients. PURPOSE: To evaluate effects of high-dose therapy and autologous stem cell transplantation (AHCT) on selected parameters of nutritional status in patients with lymphoma and to identify patients at risk of acute malnutrition post-transplant.
Methods: We evaluated outcome of 15 consecutive patients treated for lymphoma as well as selected parameters of nutritional status and searched for possible predictive factors for development of the acute malnutrition syndrome and other complications of treatment. Serum prealbumin, albumin, transferrin, and CRP levels were determined before the start of conditioning treatment, on day 0, 3, 7, 10 and 14 after transplantation. Variation of the results of nutritional markers in the period of hospitalization after AHCT in relation to the value before treatment and the correlation with the course of the post-transplant period were evaluated.
Results: Statistical analysis showed a significant reduction in the level of albumin and prealbumin on day 7 and 10 after AHCT. Transferrin levels were decreased on day 3 after AHCT, as well as on day 7 and 10. In a group of patients with BMI below 20, all patients developed diarrhea and a severe infection during post transplant period. In addition, there was a correlation between selected parameters and the occurrence of infection, fever and diarrhea.
Conclusions: Patients that undergoing AHCT are exposed to an acute condition protein-caloric malnutrition that may have an adverse impact on the course in post-transplantation period. In addition, we found that changes in concentrations of biochemical parameters at different time points in relation to AHCT may be predictive of complications during post-transplantation period. Attention should be paid to a lower concentration of prealbumin in the period prior to treatment that is correlated with diarrhea post-transplant. The nutritional status of patients may determine the risk of complications and may thus guide prophylactic interventions like nutritional therapy. Patients with reduced BMI values prior to treatment are at increased risk of complications during post transplant period.

**P623**
**Functional evaluation indicates physical losses after haematopoietic stem cell transplantation**
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Hematopoietic stem cell transplant (HSCT) procedure may cause functional losses that impair daily tasks that require physical skills. Our aim was to evaluate function of patients (pts) pre and post HSCT using an assessment in outpatient basis. From November 2008 to November 2010, 50 pts, 29 (58%) female, median age 48y (24-67), were enrolled. Collection was performed pre and post autologous or allogeneic HSCT. Instruments were 2 minutes walking test (2MWT), oxygen saturation (Sa02), heart rate (HR) and Borg Scale (BS) before and after 2MWT for gate performance evaluation; Grip Strength (GS) for strength evaluation, Schober Test (ST) for spine mobility testing and maximum and adapted activity score (MAS and AAS) of Human Activity Profile (HAP) questionnaire for function role. 50 pts were evaluated pre HSCT; 6 did not undergo HSCT; 3 died, 1 refused, and two were excluded. 44/50 (88%) underwent HSCT, 21 allogeneic, 23 autologous. 33/44 (75%) pts performed both evaluations, 11/44 (25%) pts did not: 9 died and 2 were excluded. Among groups who performed both evaluations, we found significant lower values in the post evaluation for 2MWT ($p=0.004$), GS for right and left hand ($p=0.004$ and $<0.0001$), ST, MAS and AAS ($p<0.0001$); and higher HR ($p=0.01$) before 2MWT and Sa02 ($p=0.02$) after 2MWT. Those differences indicate decrease on aerobic conditioning before physical stress, decline of gate performance, hand strength, spine flexibility and on function role on daily activities post HSCT, showing physical losses in this population. Their intensity and specificity may conduct a better rehabilitation program on post HSCT period.

**P624**
**Muscle mass change in recipients undergoing allogeneic haematopoietic stem cell transplantation**
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Backgrounds: As the precipients of allogeneic hematopoietc stem cell transplantation (allo-HSCT) become physically damaged from high dose chemotherapy or total body irradiation before HSCT and prolonged bed rest, it has been considered that rehabilitation is important to prevent disuse syndrome. Most of previous reports about physical function after HSCT were about lower extremity strength or endurance. However, there were few reports about muscle mass change which could

be affected for muscle strength or endurance after HSCT so far. Here we analyzed about body composition and changes of muscle mass in each parts of muscle after allo-HSCT.
Patients and Methods: There were 16 patients (M: 10, F: 6) who had been estimated about physiological functions after allo-HSCT between February 2010 and July 2011 in Imamura Bun-in Hospital. Median age of patients was 58 (range: 28-65) yrs. Physical therapies, which were consisted 20 to 40 minutes per day and performed 5 days a week, were carried out by physical therapists. Physiological functions wear evaluated by 6 minute walking distance (6MD) and handgrip. Body composition was evaluated using a bioelectrical impedance analysis (BIA). Evaluations were carried out at the timings of 2 weeks before allo-HSCT and after neutrophil recovery. We statistically analyzed about changes of physical strengths and body compositions.
Results: Median time to evaluate after allo-HSCT was 39 (range: 18-70) days. Change rates of physical strength before and after were -2.1% of 6MD and -8.6% handgrip respectively. This results indicated 6MD maintained but handgrip ($p=0.03$). Change rates of body compositions were -2.9% of total body muscle mass, -4.9% upper extremity muscle mass (-5.8% upper arm, -3.4% forearm), 2.7% lower extremity muscle mass (-1.1% femur, 10.5% lower leg), -6.2% trunk muscle mass, -10.2% fat body mass, -2.6% lean body mass, -4.6% bone mass, -2.6% body water volume, and -4.1% body weigh respectively. Upper extremity muscle mass ($p=0.03$), trunk muscle mass ($p=0.04$), body weight ($p<0.01$), fat body mass ($p=0.02$), lean body mass ($p<0.01$) were significantly reduced after allo-HSCT respectively.
Conclusions: It should be considered to prevent reduction of proximal muscle mass would be important in allo-HSCT recipients.

**P625**
**Quality of life in patients with haematological malignancies before and after haematopoietic stem cell transplant in a single-centre experience**
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Introduction: Hematopoietic stem-cell transplantation (HSCT) is an established form of treatment for many patients with severe disorders of the hematopoietic system. The procedure is associated with substantial morbidity and mortality, and has an enormous impact on the lives of transplant recipients and their families.
Aim: evaluating of health related quality of life (HRQoL) in allogeneic and autologous recipients that can be a useful tool in assessment of treatment side effects early and late complications and comorbidities.
Material and Methods: HRQoL questionnaire was prepared for all transplanted patients. A total of 45 patients were included for assessment during 2010-2011 (13 patients treated with allogeneic HSCT and 32 with autologous HSCT). EORTC QLQ was used as a questionnaire for assessment, consisted of 29 items and 6 multi item scales and 8 single items concerning risk factors, age, comorbidity, conditioning, support, GVHD, emotional, social and physical functioning. The patients were assessed 1, 3, 6 and 12 months posttransplant.
Results: One year EFS was 71% in auto and 81% in allo recipients respectively. The incidence of acute and chronic GVHD was 38% and 31%. HRQL showed improvement from beginning to 12 months after transplant (55,6 *vs.* 64,9 $p=0,05$). Patients experienced better QoL concerning posttranplant elevation of Hb>10,0 g/dL from beginning to 12 months after transplant (48% *vs.* 92%). We observed skin problems mainly up to 3 months after transplant and also in gastrointestinal scale ($p=0,03$). We didn't observe significant differences in other parameters during 1 tear after transplant. Finally 20 patients (45%) have returned to the previous occupational activities.
Conclusion: Guidelines for posttransplant assessment after allo and auto transplantation have not yet been developed. Assessment of HRQoL showed to be an effective in the longitudinal long term patient follow up.

**P626**
**Improvement of patients' comfort and optimisation of health care organisation by switching from IV busulfan 4 daily infusions to a once-daily administration scheme in adult population receiving conditioning therapy prior to allo HSCT**
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Introduction: IV busulfan (IV Bu) administration scheme is 0,8 mg/kg as a 2-hour infusion every 6 hours (4QD), over 4 consecutive days prior to conventional HSCT in adults.
Administration scheme with fludarabine as 3,2 mg/kg, 3 hour-infusion, once daily (QD) over 4 days has been assessed demonstrating both schedules were similar in terms of pharmacokinetics, without increased or unexpected additional toxicity.
Objective and Method: The objective is to assess for QD IV Bu compared with 4QD in adults prior HSCT, the impact on health care organisation, safety of preparation/administration and patients' comfort as perceived by caregivers.
Geographic scope includes France, Germany, Italy, Spain, UK, and Poland.
In each country, 1/2 BMT units having more than 10 HSCT per year, with prior use of 4QD IV Bu and current QD use were selected.
Clinicians, nurses and pharmacists have been identified as key stakeholders involved in the overall management of conditioning therapy. In each centre, they have been interviewed face to face during 4th Quarter 2011. 7 centres have already been interviewed and 3 are planned.
Preliminary key findings: Conditioning protocols are homogenous across countries despite slight local specificities.
Main decision maker to switch from 4QD scheme to QD IV Bu is haematologist. Main drivers of the decision are publications showing similar efficacy and toxicity for both schemes and the opportunity to get 'smoother organisations' in pharmacies and BMT units. Few barriers are mentioned except that QD is unlabeled.
Overall perception of QD versus 4QD IV Bu is very positive. Globally, stakeholders evaluate both options equivalent in terms of efficacy, perceive a better safety with QD IV Bu, a significantly better convenience and improvement of patients' comfort.
It reduces the risk of errors and staff exposure during preparation, avoids complications for patients related to repeated infusions (e.g. infections) and constitutes a significant benefit for patients avoiding night infusions with stress and anxiety.
Switching from 4QD IV Bu to QD IV Bu significantly impacts on health care organisation. With QD, IV Bu preparation is better integrated in the global management and usual timelines of other anti-cancer drugs in the pharmacy and in BMT units.
For nurses, QD IV Bu contributed to smoother timings of overall conditioning therapies administration. In addition, it reduces the needs of in-ward preparation to palliate the unavailability of teams in central pharmacies during the week-end.
Time spent to prepare and administer IV Bu once daily was significantly reduced: >1 hour nursing time and about 40 minutes pharmacy technician time saved per patient and per day, respectively 70% and 60% time savings. In addition, QD IV

Bu reduces the use of small medical devices and concomitant drugs (e.g. anti-emetics, parenteral nutrition, anxiolytics). This leads to substantial financial savings.

**P627**

**The incidence of DMSO side effects among autologous recipients with lymphoid malignancies: does the conditioning matter?**

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Introduction: DMSO is one of the contributors for morbidity during transplant procedures with cryopreserved stem cells. The safety profile of DMSO is still to be evaluated, concerning the concentration used in the freezing procedures, as well as the amount during infusion in the transplant setting.
Aim: To evaluate the incidence of DMSO side effects among autologous recipient with lymphoid malignancies in a single center experience of infusion of cryopreserved autologous stem cells with 10% and 5% DMSO solutions.
Material and Methods: A total of 81 patients with lymphoid malignancies have been evaluated for DMSO related toxicity during the autologous transplant setting, 32 myeloma patients, 19 with lymphoma, 30 patients with Hodgkin disease. The frozen cell concentration was limited in the cryopreservation bags up to 1x10(8)/ml. DMSO was not washed out from the preserved grafts and was administered through a filter in the infusion line. The returning of the frozen cells was performed in split doses depending on the amount of the graft. The upper limit in the amount of DMSO given per day was 1 g/kg.
Results: PBSC autologous grafts were 224ml in volume (62-600) before freezing, median 480,66ml (140-1000) after freezing, MNC number 3,39x10(8)/kg (1,4-7,5), CD34+cells/kg median 2,5 x10(6)/kg (1,7-3,8) viability of 74% (20-90), median 42 days (8-150) of storage. Statistical analysis of DMSO toxicity revealed moderate to mild reactions in the lymphoma group, nausea or vomiting and hypo or hypertension with the incidence of 1,2%. In the myeloma group the incidence was significantly higher 2,7%. The odds ratio (OR) in the myeloma patients was 1,41 (0, 89-3,07) and the lymphoma patients had OR 2, 9 (1,12-5,89).
Conclusion: The incidence of DMSO related toxicity is higher among myeloma patients that received melphalan conditioning. Does the dose of melphalan have its influence on the severity of this side effects is a question that has to be evaluated in a randomized study. Strategies to reduce DMSO exposure are still to be analyzed and can avoid some of the complications of autologouse transplantation.

**P628**

**Pre-engraftment syndrome after stem cell transplantation is associated with early complications, such as acute graft-versus-host disease, veno-occlusive disease, and intracranial haemorrhage**

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Background: Pre-engraftment syndrome (PES) after stem cell transplantation (SCT) remains poorly characterized, and the prognosis and appropriate management are unclear. Therefore, we retrospectively analyzed the incidence, manifestations, and clinical outcomes of PES in SCT recipients, who had received SCT at the Department of Pediatrics Hokkaido University.
Patients and Methods: We reviewed medical records of consecutive 186 child SCT recipients between April 1996 and September 2011. Indications for SCT were: hematological malignancies in 106 patients, bone marrow failure in 27, solid tumor in 35, congenital immunodeficiency in 14, and congenital metabolic diseases in 4 patients. Preparative conditioning regimen consisted of myeloaberative conditioning (MAC) was provided to 163 patients, and non-myeloabelative (NMAC) was to 23 patients. Eighty-eight patients received prophylaxis against graft-versus-host disease (GVHD) with cyclosporine (CsA) or tacrolimus (FK) in combination with methotrexate, and fifty-four patients received CsA or FK with methylprednisolone. PES was defined with noninfectious fever and skin rashes with or without the evidence of fluid retention and/or respiratory distress occurring before three days of neutrophil engraftment; an absolute neutrophil count of $>0.5x10^9$/l.
Results: PES developed in 29 of the 104 allo-BMT/PBSCT, 32 of the 53 allo-CBT, and 5 of the 29 auto-SCT patients. In allo-SCT, the incidence of acute GVHD following PES was significantly higher than in the non-PES group (p=0.016). In allo-CBT, the incidence of PES in the patients receiving MAC was significantly higher than in those receiving NMAC (p=0.035). Veno-occulusive disease (VOD) and intracranial hemorrhage (IH) were recognized in only the PES group, and the incidence of VOD in the PES group was significantly higher than that in the non-PES group in auto-SCT patients (p=0.005). Corticosteroids were required in 13 patients, and one patient died due to PES. There was no significant association between the development of PES and the donor engraftment, the speed of neutrophil recovery, non-relapse mortality, virus reactivation, or survival.
Conclusion: PES was more common after CBT. Although the differentiation with PES and early onset GVHD was difficult, corticosteroid therapy should not hesitate because virus reactivation did not have significant difference, and PES was associated with early severe complications of SCT such as VOD and IH.

**P629**

**Clinical characteristics of pre-engraftment syndrome after unrelated cord blood transplantation**

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Objectives: Pre-engraftment syndrome (pES) is a poorly defined clinical entity following hematopoietic stem cell transplantation. To characterize pES, we retrospectively analyzed the incidence, risk factors and clinical outcomes of pES in unrelated cord blood transplantation (CBT) recipients.
Methods: Data of 379 patients who received unrelated CBT were collected from 18 medical centers in Korea between October 1996 and March 2011. pES was defined as unexplained fever >38.3°C not associated with infection, and/or unexplained skin rash with or without the evidence of fluid retention occurring before neutrophil recovery.
Results: Transplant characteristics were as follows: median age 7 years; median weight, 23.5kg; 58.0% male; 41.2% double-unit CBT; 83.1% hematologic malignant disease; and myeloablative regimen in 68.3%. pES developed in 99 patients (26.1%) at a median of 6 days after transplant. Clinical manifestations associated with pES included fever (93.9%), skin rash (81.8%), diarrhea (29.3%), weight gain >3% (27.3%), pulmonary edema (13.3%), and central nervous system symptoms (8.0%). The incidences of pES in hematolgic malignant diseases and nonmalignant diseases were 25.4% and 29.7%, respectively. Of the 99 patients who developed pES, 75 patients (75.8%) received intravenous corticosteroid at a median dose of 1 mg/kg/day. Approximately 95% of patients who received corticosteroid therapy due to pES showed clinical improvement. On multivariate analysis, significant risk factors for developing pES included myeloablative conditioning, low risk disease, absence of *in vivo* T-cell depletion, infused CD34+ cells > $2.94x10^5$/kg, and infused nucleated cells > $5.94x10^7$/kg. Patients with pES showed higher incidence of infectious complications within 100 days after CBT compared to patients without pES (p=0.02).

Factors associated with graft failure were nonmalignant disease (p=0.03) and absence of pES (p<0.01). Cumulative incidence of grade II-IV acute GVHD in patients with and without pES was 56.7% and 34.2%, respectively. In multivariate analysis, the only variable associated with developing grade II-IV acute GVHD was pES (p<0.01). However, pES was not associated with chronic GVHD, treatment-related mortality at day 180 after CBT, relapse and overall survival.
Conclusion: pES showed favorable impact on engraftment. However, pES was associated with increased risk of infectious complications and grade II-IV acute GVHD after CBT.

**P630**
**Peri-engraftment syndrome in paediatric double-unit cord blood transplantation**
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Introduction: Engraftment syndrome (ES) is an inflammatory condition during neutrophil recovery following hematopoietic stem cell transplantation (HSCT) characterized by noninfectious fever, skin rash, noncardiogenic pulmonary infiltrates. But data of ES in pediatric double-unit cord blood transplantation (DUCBT) are scarce. Because of unique engraftment kinetics of DUCBT, distinctive patterns of ES could be defined as peri-engraftment syndrome (peri-ES).
Patients and Methods: We retrospectively analyzed the clinical records of 298 patients (355 transplantations) who underwent autologous or allogeneic HSCT from October 2004 to April 2011. Diagnosis of peri-ES was established by the presence of all three major criteria or two major criteria and one or more minor criteria suggested by Spitzer, excluding 'within 96 hours of engraftment' criteria.
Results: Of 298 patients (355 transplantations), 32 patients (32 transplantations) developed peri-ES with the estimated cumulative incidence of 9.0%. High cumulative incidence (56%) was shown in patients who had undergone a DUCBT (27 of 48 patients, p<0.05). The mean onset day of peri-ES of DUCBT cohort was day -5 (range, day -28 to 17) from neutrophil engraftment with mean duration of 4.5 days long (range, 1 to 22 days). In a univariate analysis and multivariate logistic regression analysis in DUCBT cohort, use of TBI (p=0.032 and p=0.043, respectively) was identified as risk factor predisposing for the development of peri-ES. However, the total infused CD3+ and CD34+ cell count were not statistically significant risk factors. There was no association between the development of peri-ES and aGVHD, cGVHD, VOD, time to neutrophil engraftment. With a median follow-up of 4.3 years, disease-free survival (p=0.51), overall survival (p=0.53) and non-relapse mortality (p=.993) did not differ between peri-ES group and the other in DUCBT cohort.
Conclusions: Not only neutrophil engraftment, but immune reactions within two cord blood units might contribute to the development of ES. So the concept of peri-ES could be applied to the patients who underwent DUCBT, particularly. Conditioning regimen with TBI was identified as a significant risk factor predisposing for peri-ES in DUCBT cohort. Further study about the pathophysiology of peri-ES in DUCBT is warranted.

**P631**
**Incidence and outcome of thrombotic microangiopathies after haematopoietic stem cell transplantation**
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Thrombotic microangiopathies (TMAs) are rare but serious complications of allogeneic hematopoietic stem cell transplantation (HSCT). This syndrome associates microangiopathic hemolysis with renal and/or neurologic impairment. The relation between allogeneic HSCT and endothelial injury are complex. To examine this association, we retrospectively studied 1,503 allogeneic patients (median age 48, range 13-73 years) between January 2002 and September 2011. Among these patients, 1028 (68.4%) developed acute graft-versus-host disease (aGvHD) (523 pts (38.4%) had a severe aGvHD of grade ≥ II). We followed 18 patients (12 male, 6 female) in whom concomitant presence of megakaryocytic thrombocytopenia (<25/nl), Coombs´-negative hemolytic anemia (<10 g/dl), absent or reduced plasma haptoglobin levels, fragmented erythrocytes and increased serum level of lactate dehydrogenase (LDH) with no other identifiable cause for those abnormalities, characteristics for TMA. Sepsis and autoimmune hemolysis were excluded. The median age at transplant was 45.5 (range, 19-60) years. Onset of TMA was at day 29 range, 15-48) after HSCT. Median level profile of enzymatic activity of the plasma metalloprotease (ADAMTS13), von Willebrand factor (VWF): AG and VWF: multimers showed not significant difference in the group of TMA versus controls without TMA. TMA had a higher prevalence in older adults and in male patients. TMA occurred in 94% in patients, who were transplanted with G-CSF mobilized and peripherally collected stem cells (PSC), but in only 6% in patients with bone marrow (BM) source, comparing all transplanted patients (1342 PSC, 161 BM). Stem cell grafts from matched unrelated donor (67%) versus related donors were associated with a higher risk of TMA. Severe aGvHD of grade ≥ III occurred more frequently in the groups with TMA (10/18 *vs.* 523/1503), and extensive chronic GvHD (28%) was ascertained in TMA patients *vs.* 19.3% in all transplanted patients. All patients with TMA died from multiple organ failure. In conclusion, our evaluation confirms a strong association between G-CSF mobilized PSC, severe aGvHD, unrelated donors and the development of TMA.

**P632**
**The successful treatment of transplant-associated thrombotic microangiopathy with eculuzimab**
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A 51 year old lady with stage IV diffuse large B cell lymphoma previously treated with RCHOP and RESHAP resulting in a partial response was admitted for sibling allogeneic transplant. She deteriorated clinically at day 34 and developed a rash and abnormal liver function and was diagnosed with grade 2 graft-versus-host disease which was treated with high dose steroids.
Concurrently it was noticed that her renal function was deteriorating with a falling platelet count and a high LDH. A blood film showed red cell fragments, spherocytosis and polychromasia. She was diagnosed with transplant associated thrombotic microangiopathy (TA-TMA).
Her calcineurin inhibitor was stopped and plasma exchange was started. This was stopped after 6 days due to poor response and a high ADAMSTS13 level of 41%. Her renal function deteriorated further and she was started on haemodialysis.
Due to previously reported benefit in renal transplant patients with TA-TMA Eculuzimab was obtained on a compassionate use basis and the patient received a dose of 900 mg weekly for 4 weeks. Within 2 weeks of starting her treatment with Eculuzimab she was dialysis independent with a 50% reduction in the number of fragments seen on her blood film. Her platelet count has remained low and a repeat marrow has shown hypoplastic changes with no evidence of lymphoma.
We describe for the first time the successful treatment of TA-TMA with Eculuzimab. Given the poor prognosis of this condition this treatment may offer new hope to transplant patients who develop this rare but life threatening complication.

**P633**

**Intravenous versus oral busulfan administration results into a dramatic reduction of veno-occlusive disease (VOD) incidence in a randomised trial assessing fresh frozen plasma+heparin versus heparin-alone as anti-VOD prophylaxis**

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We studied prospectively, risk factors and VOD-incidence in relation to anti-VOD prophylaxis, in 336 patients who consecutively underwent hematopoietic cell transplantation (HCT) between 3/2004-3/2011. Patients were randomized to receiving Heparin (Group A) or 2fresh-frozen plasma (FFP)/day+Heparin (Group B). FFP administration during conditioning was based on our retrospective study in which FFP+Heparin reduced VOD, probably by minimizing the drop of natural anticoagulants post conditioning. AT/ProteinC levels were measured per week up to day 28. Patients with median age 36(5-68) years underwent autologous (n=152) or allogeneic (sibling=117, volunteer=61, alternative=6) HCT from blood (n=315), bm (n=19) or cord blood (n=3) following mainly, myeloablative conditioning (292/336). The majority were transplanted at advanced disease and 24 patients twice. In groupA were randomized 164 and in groupB 172 patients with similar baseline characteristics. The cumulative VOD-incidence was 2% (7/336, severe:5/7, moderate:2/7), the VOD-associated mortality 57% and the day-100 mortality 70% (vs 6% in no-VOD patients, p<0.0001). Anti-VOD prophylaxis didn't influence VOD incidence (Group A:3/7, Group B:4/7). All patients with lethal VOD had more than one known risk factors. In Busulfan (BU)-conditioned patients, VOD-incidence was 50% (3/6) and 2.5% (3/119) with oral and iv administration respectively, without difference in VOD-induced mortality. Univariate analysis demonstrated significant correlations between VOD and diagnosis, AT/ProteinC levels (d14 and 21), use of hepato-nephrotoxic drugs, baseline liver status, conditioning, mode of BU administration. Multivariate analysis demonstrated the allo-HCT (95%CI, OR:14.1, p=0.04 *vs* auto-HCT) and oral BU (95%CI, OR:75.4, p<0.001 *vs* iv BU) as factors significantly associated with VOD. We observed significant reduction in Alpha Tau /Prot-C levels as compared to baseline, both in VOD- (AT:d14-28/Prot-C:d21-28, p≤0.03) and no-VOD-patients (AT:d7-21/Prot-C:d0-21, p≤0.00002). However, VOD-subjects had lower Alpha Tau (d14-28) and Prot-C(d0-d28) values *vs* no-VOD individuals (p≤0.02). Overall, the improved pharmacokinetics of iv BU leads to a dramatic decrease in VOD incidence. The drop of natural anticoagulants occurs independently of VOD and their higher reduction at VOD rather represents an epiphenomenon. Replenishment with FFP, although it may correct the anticoagulant values, it cannot stem the syndrome's progression.

**P634**

**Haematopoietic stem cell transplantation-associated thrombotic microangiopathy – severe complication. A single-centre experience**

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Introduction: Thrombotic microangiopathy (TMA) is a severe complication of allogeneic haematopoietic stem cell transplantation (allo HSCT) recognizing multiple risk and trigger factors and having in the majority of the cases a dismal outcome. The incidence of TMA is rather difficult to establish due to diagnostic criteria which often overlap with those defining other concomitant co-morbidities.

Objectives: The aim was to assess the incidence, risk factors, triggers, management and outcome of TMA in patients with allo HSCT transplanted in the Regional Center for Bone Marrow Transplantation Timisoara/Romania.

Patients and Methods:. This retrospective study was performed on a cohort of 38 patients consecutively receiving a matched related or unrelated donor allo HSCT after myeloablative conditioning in our centre between 2003-2011. All patients received post-transplant immunosuppression with cyclosporine and short course methotrexate. The diagnostic criteria were in accordance with the Blood and Marrow Transplant Clinical Trials Network consensus or the International Working Group consensus. Graft-versus-host disease, infections and cyclosporine toxicity were considered as possible triggers of TMA, if present at diagnosis or occurring at maximum 1 week prior to the onset of TMA.

Results: Five years projected overall survival (p OS) for the whole studied cohort was 53.4 %. Four cases, representing 10.5 % of all patients, fulfilled the diagnostic criteria for transplant-associated TMA. Median time of onset of TMA was 23 days post-transplant. Possible factors triggering TMA were cyclosporine toxicity in one case and sepsis in the other three cases. All patients with TMA developed renal impairment and three out of four developed neurological dysfunction. After diagnosis, cyclosporine was reduced or temporarily stopped. Treatment of TMA was supportive. One patient responded to treatment and is alive and disease-free at 39 months post-transplant. The other three patients died of multiorgan failure at a median time of 20 days after diagnosis of TMA.

Conclusion: The incidence of transplant - related TMA was similar with that reported in literature although its assessment may be very difficult if other severe co-morbidities are present such as severe infections. In our patients TMA was associated with high mortality, only one patient responding to reduction of cyclosporine, confirming the possible toxic cause for this complication.

**P635**

**The development of haemophagocytic syndrome is associated with high-risk disease and viral reactivation and lower day 100 survival**

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The activation of macrophages in the bone marrow early in the post-transplantation period, which often results in hemophagocytic syndrome (HPS), is a previously unrecognized complication with a negative impact on the outcome of allogeneic hematopoietic SCT (AHSCT). We retrospectively analyzed the relationship between HPS and bacteremia/viremia after AHSCT, as well as its impact on subsequent outcome.

We reviewed 71 consecutive patients who received AHSCT between July 2008 and July 2011 at our institution. Multiplex PCR was performed weekly from day 0 of AHSCT on all patients to detect a fixed set of viruses. Diagnostic criteria of AHSCT-associated HPS are as follows; fulfillment of two major criteria (histological evidence of bone marrow hemophagocytosis and impaired engraftment after AHSCT) or one major and all four minor criterion (high grade fever, hepato-splenomegaly, elevated ferritin, and elevated serum lactate dehydrogenase value). This study was approved by the institutional review board.

The median age was 52 (range, 17-69) years. Diagnosis included myeloid malignancy (n=43), lymphoid malignancy (n=27), and benign hematologic disease (n=1). Stem cell sources were

bone marrow (n=52), peripheral blood (n=8), and cord blood (n=11). 34 patients had high risk diseases. 39 patients received myeloablative conditioning regimens. HLA was matched in the Graft-versus-host direction in 44 patients. Among 71 patients, 8 developed HPS (HPS group) at a median of 21 days after AHSCT and died because of graft failure (n=2), thrombotic microangiopathy (n=2), infection (n=3), or intracranial hemorrhage (n=1). Multivariate analysis demonstrated that the development of HPS had a significantly worse impact on day 100 survival after AHSCT. {HPS group 50 % and non-HPS group 89 %, p<0.01}. In the HPS group, 6 experienced viremia (4 with CMV, 2 with EBV) shortly before the occurrence of HPS. EBV was not detected in the non-HPS group. Although CMV viremia was also occasionally observed in the non-HPS group, the copy number of CMV detected was considerably higher in the HPS group. When background characteristics were statistically compared, only high-risk disease was associated with the development of HPS.
The development of HPS considerably increased therapy-related mortality and lowered day 100 survival. The development of HPS associated with high-risk disease and the reactivation of EBV or CMV may contribute to its development in a considerable proportion of patients.

**P636**

**Late-onset haemorrhagic cystitis after haploidentical haematopoietic stem cell transplantation in patients with advanced leukaemia: ATG matters**

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Objectives: The cause of late-onset hemorrhagic cystitis (LOHC) after allogeneic hematopoietic stem cell transplantation (HSCT) has remained obscure.
Methods: We limited our analyses to patients with advanced leukemia underwent a haploidentical HSCT and treated with ATG–contained regimens to define the influence of different doses of ATG in conditioning on LOHC.
Results: From January 2003 to February 2011, we enrolled 75 patients in this retrospective study. Thirty-nine patients transplanted before June 2008 were treated with high-dose ATG (10 mg/Kg), whereas subsequent 36 patients received low-dose ATG (6 mg/Kg) in conditioning. There was no significant difference between the two groups in engraftment, survival, leukemia relapse and non-relapse mortality, GVHD and cytomegalovirus reactivation. However, LOHC occurred in the 16.7% of patients receiving low-dose ATG and 38.5% of patients receiving high-dose ATG (P=0.027). High-dose ATG in conditioning, male sex, early donor lymphocyte infusion and cytomegalovirus reactivation were significant risk factors for LOHC. However, only the high-dose ATG in conditioning (HR 2.96, 95% CI 1.143-7.663, P=0.025) and male sex (HR 4.033, 95% CI 1.355-12.008, P=0.012) are independent risk factors on the multivariate analyses.
Conclusion: LOHC was more prevalent in the male patients and recipients of high-dose ATG in conditioning in the setting of haploidentical HSCT.

**[P636]**

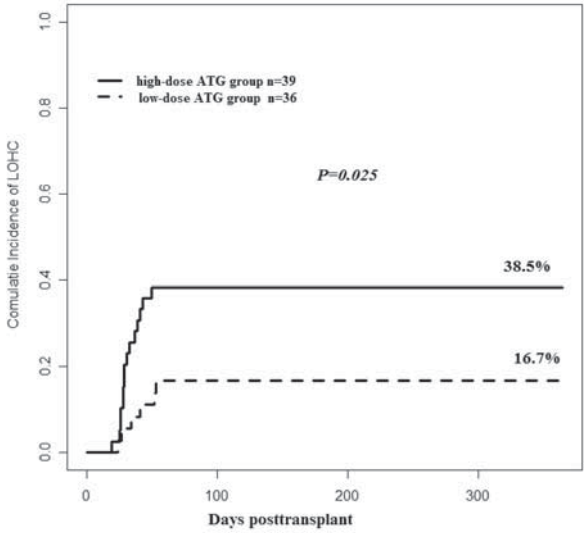


Figure 3A. The influence of ATG dose in conditioning for LOHC

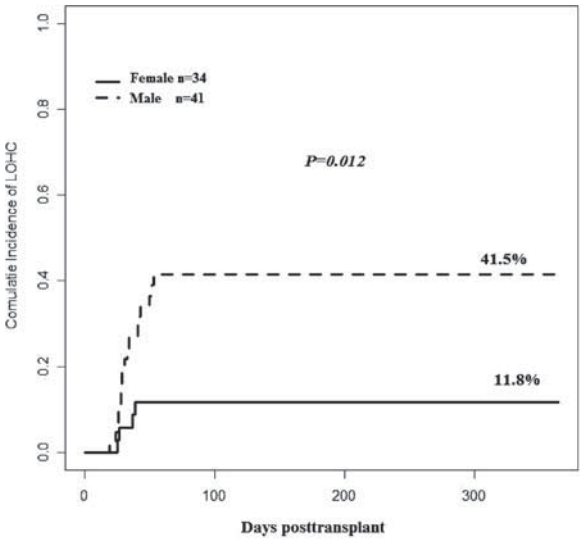


Figure 3B. Patients' gender for LOHC

**P637**

**Respiratory complications in paediatric stem cell transplant recipients: could a high-resolution computed tomography score aid in diagnosis or prognosis?**

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Background: Hematopoietic stem-cell transplantation (HSCT) can be complicated by a variety of life-threatening infectious and non-infectious pulmonary complications. Differentiation between these entities is of outmost clinical importance. High-resolution computed tomography (HRCT) abnormalities may substantially overlap and whether HRCT can differentiate between infectious and non-infectious complications is not well-known.
Aims: First to develop a HRCT-score for patients with respiratory complications post-HSCT. Secondly to assess the

reproducability of this HRCT score and, and its place in differentiation between infectious and non-infectious lung disease including allo-immune lung syndrome (allo-LS). And finally to assess the association of this HRCT- score with disease severity.
Methods: Consecutive HRCT scans of the chest in 45 pediatric HSCT recipients with respiratory symptoms and/or suspicion for infection were scored by two independent radiologists for various airway and parenchyma abnormalities. Clinical diagnosis was established after the disease episode resolved by an expert physician who had access to all available information. Maximum disease severity during the disease episode was graded from 0 (asymptomatic) till 4 (death).
Results: HRCT scoring was reproducible for the composite HRCT-score and all items, except airway wall and septa thickening. Abnormalities overlapped between infectious and non-infectious complications, but the severity score was significantly higher in all allo-LS for HRCT-composite, HRCT-ground glass (only for early allo-LS), HRCT-bronchiectasis (late allo-LS) an HRCT-air trapping (late allo-LS) when compared to infectious disease. The composite HRCT-score predicted maximum disease severity, a 10-points (scale 0-100) increase in composite HRCT score corresponded to a severity score increase by 1.
Conclusion: Our data suggest that while abnormalities overlap between infectious and non-infectious complications in pediatric HSCT recipients a HRCT severity score aids in the differentiation and predicts the prognosis of the pulmonary disease episode.

**P638**

**Pulmonary complications in haematopoietic stem cell transplant patients - a single-centre experience**

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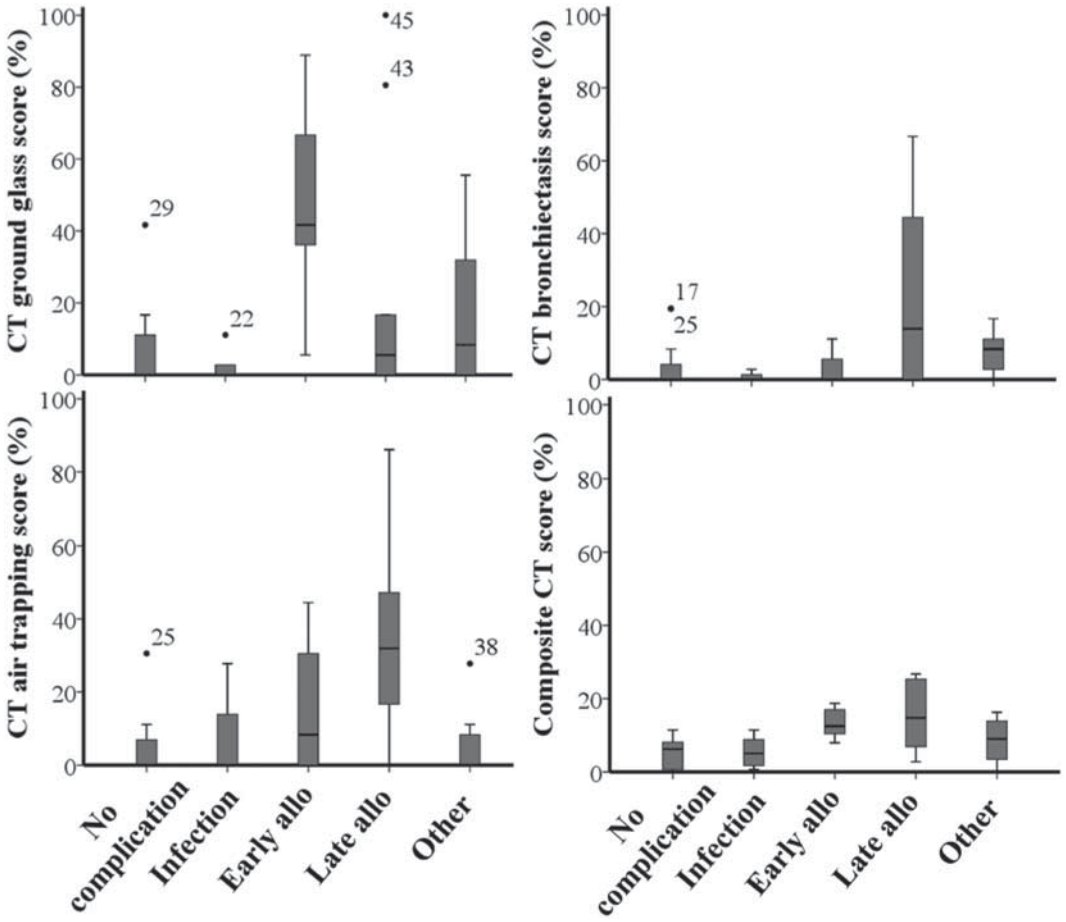
Background: Pulmonary complications are the most probable causes of life-threatening episodes in hematopoietic stem cell transplantation patients. There are a variety of causes including infectious and non-infectious etiologies to result in those events. We analyzed the experience of our institution to treat such kind of patients.
Methods: For all patients after the treatment of hematopoietic stem cell patients once developed respiratory symptoms, we performed plain film X ray and CT scan of lungs and prepared bronchoscopic examination including transbronchoscopic biopsy and bronchioloalveolar lavarge, and transcutaneous core needle biopsy of lung or thoracoscopic resection of lung tissue to check cytology, histopathology, and sent for bacterial, fungal, and tuberculous cultures and special stains for bacteria, fungus, tuberculosis, CMV, HSV, PJP, and PCR for CMV, HSV, PJP, RSV, influenza, parainfluenza, adenovirus, and VZV.
Results: Between March 2001 and November 2011, we have 181 patients undergoing hematopoietic stem cell transplantation at our institution including 108 patients received allotransplant and 73 autotransplant. Forty-five episodes of pulmonary complications occurred in allogeneic stem cell transplant except one invasive pulmonary aspergillosis occurred in an

**[P637]**



autotransplant patient (24.8% in all patients or 40.7% in allotransplant patients). For allogeneic stem cell transplantation patients, the incidence of the diverse etiology of pulmonary complications are as follows: CMV pneumonia 11.1%, chronic GVHD or bronchiolitis obliterans 7.4%, capillary leak or engraftment syndrome 5.6%, fungal pneumonia 3.7%, tuberculous pneumonia 3.7%, unspecified pneumonia 3.7%, pulmonary hemorrhage 2.8%, RSV pneumonia 1.9%, HSV pneumonia 0.9%, and leukemic infiltrate 0.9%. Moreover, the mortality rate of these pulmonary complications were up to 46.7% (21 patients in these 45 episodes).
Conclusions: Post-transplant pulmonary complication is a life-threatening event and the two most probable causes are CMV pneumonia and chronic GVHD related bronchiolitis obliterans. It is crucial to make early diagnosis and to deliver the most appropriate treatment for these patients.

**P639**

**Vitamin D deficiency at the time of HSCT may be safely reversed by high-dose vitamin D therapy**

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The major circulating vitamin D (VitD) is 25-hydroxyvitamin D3 (25-OH-D3), produced by ultra-violet B irradiation of 7-dehydrocholesterol in skin & subsequent hydroxylation in liver to 25-OH-D3. Further hydroxylation in kidney yields biologically active 1,25-OH-D3. VitD has immunomodulatory effects & low levels are epidemiologically associated with immune dysfunction, susceptibility to infection & malignancy. VitD therapy may ameliorate chronic graft-versus-host disease.
United Kingdom prevalence of suboptimal VitD levels, varying with season, is 61-90%. Routine assay of VitD was incorporated in the pre-allogeneic haemopoietic stem cell transplant (HSCT) evaluation at Leeds Teaching Hospitals (LTH) in 2009. 25-OH-D3 ranges (nmol/L) are defined: deficiency <30, depletion 30-50, insufficiency 50-75 and replete >75 nmol/L. Overall, 96% of patients admitted for HSCT & assayed for VitD from 01/2009-06/2010 were non-replete, with no seasonal pattern. Routine prescribing practice was daily oral Calceos (50 mmol calcium & colecalciferol 800 international units (IU)).
Cohort 1 (01/2009-06/2010): VitD assayed pre-HSCT in 47/55 indicated 40% deficient, 33% deplete, 11% insufficient & 2% replete. Following regular Calceos therapy, VitD, measured at 3 months (m) post-HSCT, rose by a median of 9% (range 56-249) overall, with changes respectively of 46% (0-249), 7% (41-56) & 17% (14-20) in the deficient, deplete & insufficiency subgroups. At LTH the VitD replacement schedule in patients with osteoporosis recommends initial oral ergocalciferol 300,000 IU in VitD deficiency, 3 doses of oral colecalciferol 40,000 IU in VitD depletion & Calceos in VitD insufficiency. This therapy algorithm was adopted for cohort 2 (07/2010-07/2011).
Cohort 2: VitD was assayed pre-HSCT in 31/31 patients with 55% deficient, 23% deplete, 10% insufficient & 13% replete. Therapy was completed as per algorithm in 17/27 and VitD levels post therapy were available in 13 patients. VitD levels, measured at 3m post-HSCT, rose by a median of 58% (range 6-663) overall, with changes respectively of 198% (6-663), 44% (30-80) & 50% (26-80) in the deficient, deplete & insufficiency sub-groups. VitD therapy was not associated with toxicity in any patient. VitD deficiency states are common pre-HSCT in the UK & reversible safely & quickly with high dose VitD therapy. The adopted treatment alogorithm provides a targeted, individualised and more successful approach to VitD replacement than standard therapy.

**P640**

**Hyperglycaemia during the early phase after haematopoietic stem cell transplantation is caused by the elevation of insulin resistance**

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Introduction: Although it is well known that hyperglycemia is common after hematopoietic stem cell transplantation (HSCT), its mechanism causing hyperglycemia is still to be determined, especially during the early phase after HSCT. To explore the cause of hyperglycemia, we prospectively monitored the glycemic status.
Patients and Methods: From October 2008 to September 2009, a total of 90 patients who underwent HSCT (allogeneic n=79, autologous n=11) were enrolled in this prospective observational study. Patient characteristics are shown in Table 1. The target of glucose level was set from 80 to 150 mg/dL. The fasting glycemic status was monitored immediately before the conditioning regimen, at the day of HSCT, and weekly up to 1 month after HSCT. To assess the insulin resistance (IR), we used the homeostasis model assessment (HOMA)-IR, using the following formula: [fasting insulin (μU/mL) x fasting glucose (mg/dL)]/405.
Results: Mean blood glucose level was 94, 109, 114, 121, 122, and 117 mg/dL, and mean serum immunoreactive insulin (IRI) level was 11.1, 25.4, 28.8, 30.3, 29.3, and 23.1 μU/mL at pre-transplant, day 0, 7, 14, 21 and 28, respectively. Serum C-peptide elevated at the day of HSCT compared to the baseline, and it decreased afterward even though glucose level serially elevated (mean serum C-peptide level was 2.5, 4.1, 2.9, 3.0, 2.5, and 2.2 ng/ml at pre-transplant, day 0, 7, 14, 21 and 28, respectively). Mean HOMA-IR score was 2.6, 7.5, 8.6, 9.9, 10.3 and 7.5 at pre-transplant, day 0, 7, 14, 21 and 28, respectively. HOMA-IR significantly elevated after HSCT, which was statistically higher after allogeneic HSCT compared to autologous HSCT at day 14 and day 21 (Figure 1). Patients who received total parenteral nutrition (TPN) after HSCT had significantly higher HOMA-IR at day 7, day 14, day 21 and day 28 compared to those who did not receive TPN. There was a significant correlation between C-reactive protein level and HOMA-IR at day 7, day 14, day 21 and day 28 ($P<0.05$). Patients who received myeloablative conditioning regimen had significantly higher HOMA-IR at day 14 and day 21 compared to patients who received reduced-intensity conditioning regimen.
Conclusions: Significantly elevated IR was observed during the early phase after HSCT. Glucose level should be closely monitored after HSCT even if the patients don't have diabetes before transplant.

[P640]

Table 1 Patients characteristics (n=90)

| | | | |
|---|---|---|---|
| Age (median, range) | | 47 | (17-69) |
| BMI (median, range) | | 21.6 | (13.6-30.3) |
| Disease | | | |
| | AML | | 29 (32%) |
| | MDS | | 8 (9%) |
| | ALL | | 15 (17%) |
| | ML | | 33 (37%) |
| | MPD | | 2 (2%) |
| | MM | | 3 (3%) |
| DM | | | |
| | type 1 | | 1 (1%) |
| | type 2 | | 4 (4%) |
| Type of transplant | | | |
| | allogeneic | | 79 (88%) |
| | | myeloablative | 47 (52%) |
| | | reduced-intensity | 32 (36%) |
| | autologous | | 11 (12%) |
| Stem cell source (in allogeneic HSCT) | | | |
| | BM | | 56 (71%) |
| | PB | | 20 (25%) |
| | CB | | 3 (4%) |
| | related | | 20 (25%) |
| | unrelated | | 59 (75%) |
| HLA disparity | | | |
| | HLA matched | | 54 (68%) |
| | HLA mismatched | | 25 (32%) |
| GVHD prophylaxis | | | |
| | CSP | | 11 (14%) |
| | TAC | | 68 (86%) |
| | with MTX | | 72 (91%) |

BMI=body mass index, AML=acute myeloid leukemia, MDS=myelodysplastic syndrome, ALL=acute lymphoblastic leukemia, ML=malignant lymphoma, MPD=myeloproliferative disease, MM=multiple myeloma, DM=diabetes mellitus, HSCT=hematopoietic stem cell transplant, BM=bone marrow, PB=peripheral blood, CB=cord blood, HLA=human leukocyte antigen, GVHD=graft-versus host disease, CSP=cyclosporine, TAC=tacrolimus, MTX=methotrexate.

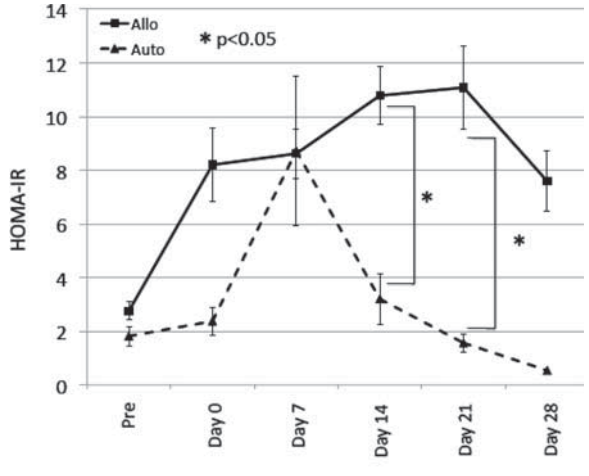


Figure 1

**P641**

**Peripheral neuropathy induced by VAD and VTD followed by high-dose melphalan with autologous stem cell transplantation and bortezomib consolidation for multiple myeloma: toxicity analysis of KMM51 study**

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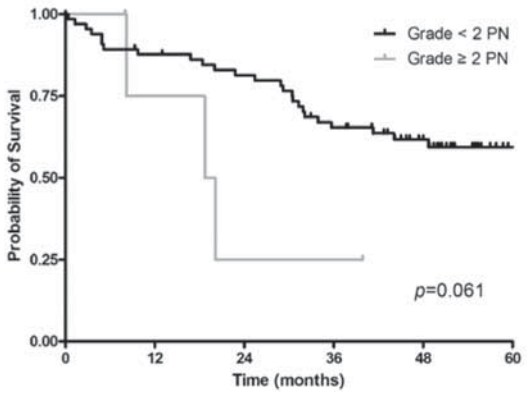
Background: Peripheral neuropathy (PN) is one of the most common adverse events during treatment of patients (pts) with multiple myeloma (MM). However, there is still a paucity of reports on clinical courses and risk factors regarding PN.

Methods: We previously performed KMM51 study using vincristine, doxorubicin, and dexamethasone (VAD) followed by bortezomib, thalidomide, and dexamethasone (VTD) as induction chemotherapy followed by high-dose melphalan with autologous stem cell transplantation (auto-SCT) and bortezomib consolidation for newly diagnosed MM (ClinicalTrials.gov NCT00378755; Kim HJ, *et al.* Ann Hematol 2011). Using toxicity data of this study, we analyzed clinical courses of PN and risk factors related to PN.

Results: A total of 71 pts were included. Median age was 57 (range, 31-64) years. The male to female ratio was 42: 29 (59.2%: 40.8%). Median follow-up duration was 52.0 (range, 3.5-63.0) months. Before study entry, pts with grade (Gr) ≥2 PN were excluded. Among 71 pts receiving VAD, Gr ≥2 PN developed in 5 pts (7.0%). Among 62 pts receiving VTD, 10 pts (16.1%) had a new-onset Gr ≥2 PN. After VTD, among 4 pts with Gr ≥2 PN induced by VAD, 3 pts maintained the same severity, and 1 pt became asymptomatic. Among 51 pts receiving auto-SCT, 3 pts (5.9%) had a new-onset Gr ≥2 PN. After auto-SCT, among 9 pts with pre-existing Gr ≥2 PN, 2 pts maintained the same severity, and 7 pts became asymptomatic. Among 42 pts receiving bortezomib consolidation, 3 pts (7.1%) had a new-onset Gr ≥2 PN. After bortezomib consolidation, among 5 pts with pre-existing Gr ≥2 PN, 1 pt maintained the same severity, and 4 pts became asymptomatic. In relation to VAD, pts with Gr ≥2 PN tended to have shorter overall survival (OS) (p=0.061; Figure 1). In relation to VTD, serum creatinine (Cr) ≥2.0 mg/dL at diagnosis was significantly associated with Gr ≥3 PN (p=0.016). In addition, Gr ≥;3 PN was significantly associated with shorter OS (p=0.021; Figure 2) along with a tendency toward a shorter progression-free survival (p=0.162). However, Gr ≥3 PN did not influence complete remission (CR) and near CR rate (p=0.636).

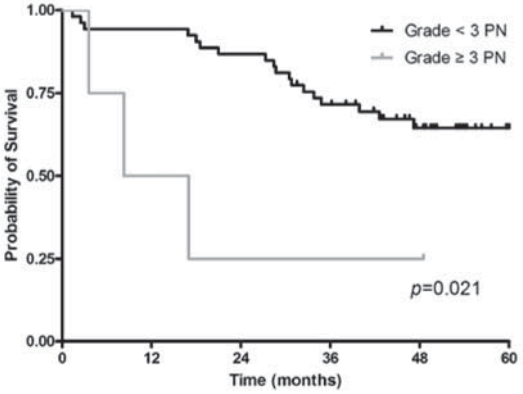
Conclusions: During KMM51 study, the incidence of PN was substantial although some of them were reversible. During VTD, serum Cr ≥2.0 mg/dL at diagnosis was significantly associated with Gr ≥3 PN. Gr ≥3 PN during VTD resulting in treatment delay and dose modification significantly influenced the prognosis of pts.

Figure 1. OS according to grades of PN during VAD chemotherapy




**[P641]**

Figure 2. OS according to grades of PN during VTD chemotherapy




**P642**

**Posterior reversible encephalopathy syndrome after allogeneic stem cell transplantation**

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Posterior reversible encephalopathy-syndrome (PRES) is a rare, but serious complication after allogeneic stem cell transplantation (alloSCT). Even though PRES is clearly characterised by the rapid development of typical neurological symptoms together with specific findings in magnetic resonance imaging (MRI), controversy about risk factors and therapeutic management is quite obvious. We retrospectively analysed data from 137 consecutive patients who underwent alloSCT in our department from January 2007 to August 2011 and identified 5 patients with PRES (3.6%, age 23 to 59 years, 3 male, 2 female). In all cases PRES was diagnosed by MRI and occurred between day +6 and day +15 after alloSCT (mean day +11). Symptoms at presentation were altered level of consciousness (n=5), seizures (n=4), headache (n=4), visual disturbance (n=3), or paralysis (n=1). Disease stadium, conditioning regimen, stem cell source, age and gender seemed not to influence development of PRES. All patients received a GvHD prophylaxis with cyclosporine (CSA) together with low-dose methotrexate (n=3) or rabbit ATG (n=2). As additional risk factors we identified a phase of hypertensive dysregulation and a systemic inflammatory reaction in all patients preceding PRES. At the onset of PRES all patients received a 2nd line antibiotic therapy based on a carbapenem together with either a glycopeptide (n=3) or linezolide (n=2). None of the patients had signs of acute GvHD preceding PRES. In 3 patients immunsuppression with CSA was stopped immediately and after bridging with mycophenolate mofetil and prednisolone the mTor inhibitor everolimus was commenced. Further applying an intensified anti-hypertensive treatment PRES was completely reversible within a few days. Interestingly, in one patient PRES was also completely reversible after medical normalisation of blood pressure even though CSA was continued. One patient developed a systemic inflammatory response syndrome and died after 3 weeks of intensive care treatment due to a multi-organ failure. In all patients with seizures the administration of levetiracetam was effective in preventing further seizures.

Conclusion: Irrespective of hypertension and CSA, a systemic inflammation and/or medication with a carbapenem might be an additional risk factor for PRES. Normalisation of blood pressure and switching the GvHD prophylaxis to the mTor inhibitor everolimus are recommended to treat PRES.

**P643**

**T depletion is associated to an higher risks of encephalitis in adults patients receiving allogeneic haemopoietic stem cell transplantation**

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Aim of study was to identify if encephalitis represents an emerging complication in patients receiving allogeneic Hemopoietic stem cells Transplantation (HSCT).
Methods: To identify the occurrence, the spectrum and the risk factors inducing neurological diseases, we carried out a retrospective cohort study including 391 adult patients followed up after allogeneic HSCT. All patients were affected by onco-hematological diseases and received transplantation between January 1997 and June 2011. One hundred nine patients received an *in vivo* T depletion by ATG or Alemtuzumab.
Results: Encephalitis was documented in 18/391 patients (4,6%) and occurred as an early complication in 10/18 pts. The time-trend of encephalitis incidence is illustrated in Figure 1, showing a fast increase since 2008. The percentage of patients receiving T depletion in the same period is also indicated. Vascular diseases were the most frequent causes of encephalitis (39%). Infectious encephalitis was reported in 11% of patients. Detected agents were represented by: CMV 1 pt; HHV6 1 pt; Aspergillus 2 pts, and Pseudomonas 1pt. Two patients developed a late toxic encephalic disease related to radiotherapy, one patient had a cerebral vasculitis and one a posterior reversible encephalopathy. The etiology of encephalitis was not identified in 3 patients. Sixteen patient affected by encephalitis died, but encephalitis was the direct cause of death in only 13 patients.
Univariate logistic regression models for potential risk factors identified T–cell depletion (OR=7.5, p=0.001), time of transplant (3-year calendar periods of transplant, linear trend between 1997-2011) (OR=2.3, p=0.001) and unrelated donor (OR=3.5, p=0.001), as significant risk factors. In multivariate logistic analysis including also disease status at transplantation, conditioning regimen and acute Graft versus Host Disease, T-depletion (OR=3.9, p=0.017) and time of transplant (OR=2.5, p= 0.002) were the only significant risk factors for encephalitis.
Conclusions: Our analysis confirms a strong association between the use of *in vivo* T cell depletion and risk of encephalitis. Moreover, the increasing time-trend in incidence of encephalitis could be associated to a more frequent use of T depletion in our Centre in the last decade.

**P644**

**Adverse drug reactions in bone marrow transplant patients**

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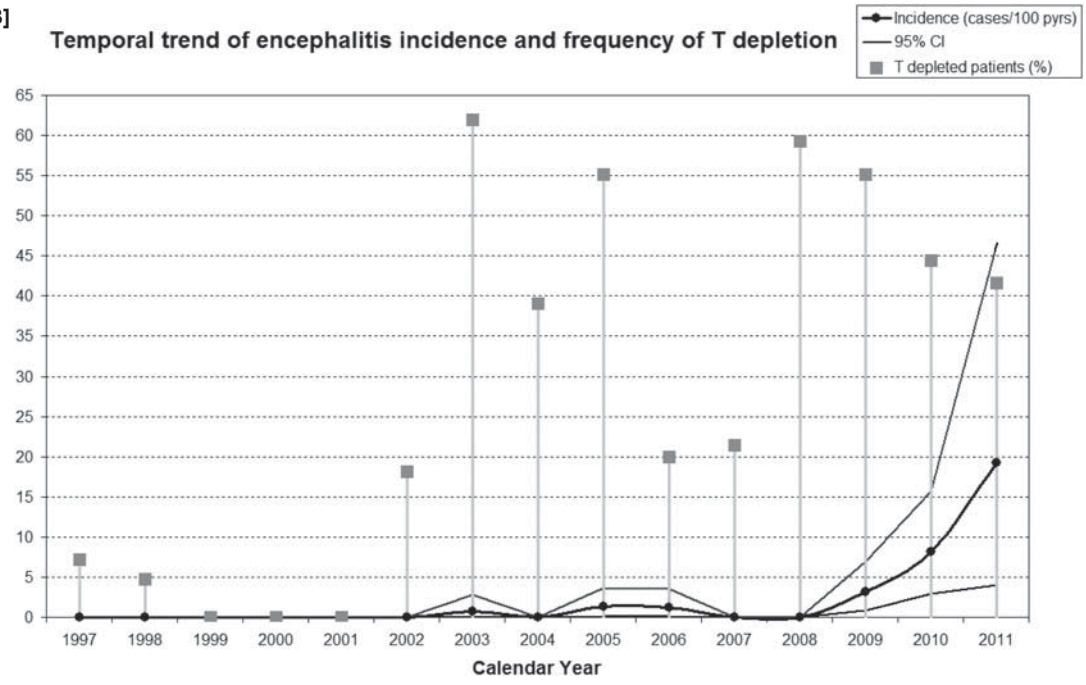
Objectives: To estimate the incidence and describe the characteristics and outcomes of adverse drug reactions (ADRs) in hospitalized bone marrow transplant (BMT) patients.
Methods: This was a prospective observational study on patients admitted to a BMT unit at a comprehensive cancer center in Jordan between October 2010 and March 2011. ADRs were defined as “any response to a drug which is noxious and unintended and which occurs at doses normally used for prophylaxis, diagnosis, or therapy of disease or for the modification of physiologic function”. The suspected ADRs were identified by the clinical pharmacist covering the unit utilizing a modified version of a trigger tool developed by the Institute of Health Improvement (IHI). Identified ADRs were verified by a BMT consultant physician. The ADRs were characterized according to medication involved, system affected, severity, and preventability. Myelosuppression was excluded since it was an anticipated and intended effect. The outcome of an ADR was determined based on the required management according to the Hartwig’s severity assessment scale.
Results: Over the 6-month period, there was a total of 118 admissions. Eighty-two of them were male patients (69.5%). The median age was 22 years (range 1– 57 years). Total number of reported ADRs was 206. Most reported ADRs were due to chemotherapy 67 (32.5%), immunosuppressants 45 (21.8%), antibiotics 39 (18.9%), Opioids 24 (11.7%) and others 31 (15.1%). The most common types of ADRs were gastrointestinal

**[P643]**



41 (19.9%), febrile neutropenia 27 (13.1%), elevated drug levels 21 (10.2%), cardiovascular 20 (9.7%) and dermatological 17 (8.3%). Of all reported ADRs there was no fatality or permanent harm to patients, however; 3 (1.5%) required ICU admission, 25 (12.1%) required increase in level of care, 124 (60.2%) required treatment, 40 (19.4%) required drug to be held and 14 (6.8%) were with no harm to the patients. Twenty-three (11.1%) of the identified ADRs were judged preventable.
Conclusion: The incidence of serious ADRs in BMT patients was found to be high. Most ADRs were related to chemotherapy and resolved upon engraftment. It is warranted to improve the ADRs reporting and analysis systems in order to develop interventions that reduce or eliminate the harm associated with the intensive use of medications in the BMT setting. This study will open the way for further research projects to explore ADRs in more specific BMT populations.

**P645**
**Early non-haematological complications after autologous haematopoietic stem cell transplantation in elderly lymphoma patients**
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Objective: Due to multi organ toxicity of conditioning regimens, early non-hematological effects can be more hazardous in older group of patients (pts) with comorbidities.
The aim of the study was to analyze the organ related complications in early, up to 30 days post-transplant period in lymphoma pts and to explore pts' age impact on incidence and grade of adverse events.
Methods: Between January 2005 and November 2011, 315 consecutive lymphoma pts underwent high dose therapy (HDT) followed by autologous stem cell transplantations (ASCT) (n=325). Median age of pts was 42 years (range 18-67). 44 pts were above 60 year old. Conditioning regimens were: BEAM (carmustine, etoposide, cytarabine, melphalan) in 216, melphalan 200 in 64, CBV (carmustune, etoposide, cyclphosphamide) in 17, cytarabine, melphalan or cyclophosphamide - in 28 pts. 32% pts above 60 year had some comorbidities: in 71 % cardiovascular.
Results: Early non hematologic complications within 30 days after ASCT were reported in 91% of pts. The most common event was neutropenic fever (70% of transplants) with septic shock in 1.9% of pts. Oral mucositis occurred in 54% of pts, with grade 3-4 in 27% of pts, 48% pts had diarrhea, 22% - abdominal pain. Prolonged, more than 7 days, nausea and vomiting occurred in 36% of pts. There was no difference in the incidence of early complications between younger (18-59 years) and older (above 60) patients (Table 1). Median hospitalization for younger pts was 22 days (range 12-61) and 21 days (range 16–38, p>0,5) for older pts. There were no toxic deaths.
Conclusions: HDT resulted in remarkable incidence of toxicity during early post-transplant period. The most common complications were: neutropenic fever, infections, oral mucositis and diarrhea. There was no difference in the incidence of toxic events between younger and older pts. Age and comorbidities did not increase the risk of serious adverse events in early post-transplant period.

**P646**
**HCT-CI correlates poorly with outcome following allogeneic stem cell transplant: impact of underlying diagnosis, patient selection and assessment of organ function**
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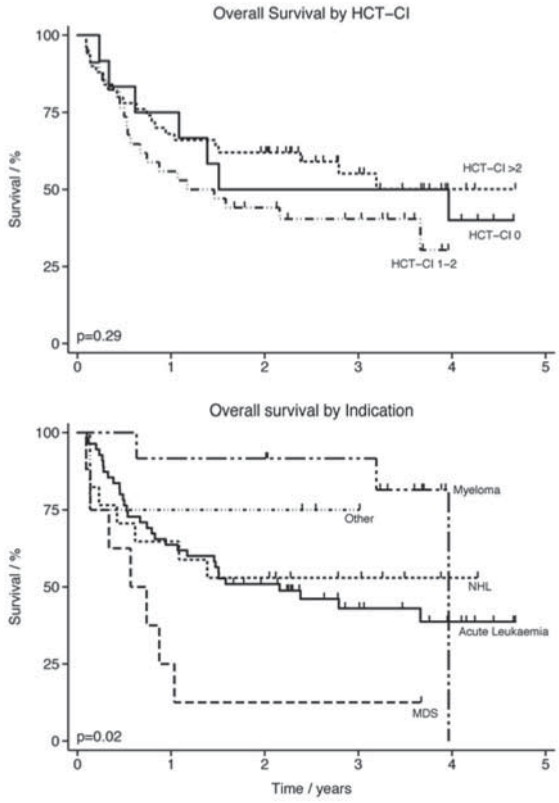
There has been considerable recent interest in the use of co-morbidity indices to risk stratify patients undergoing allogeneic stem cell transplantation (allo-SCT), although the optimal scoring system is unknown. The utility of the haematopoietic cell transplantation specific co-morbidity index (HCT-CI) is not clearly established. We report the results of using the HCT-CI in a single centre.
96 sequential patients transplanted during 2007-9 were included. Patient demographics, diagnosis and transplant procedures are summarised in Figure 1. Co-morbidities were assessed according to the HCT-CI. Clinical evaluation and blood tests were available in all patients. Cardiac function results (echocardiogram/MUGA) were available in 95 of 96 patients; respiratory function results (assessed in specialist lung function laboratories) were available in 90 of 96.
50 of 96 (52%) are currently alive at a median follow-up of 24 months (range 1-56). Day 100 and 1 year non-relapse mortality (NRM) for the entire cohort were 11.4% (95% CI 6.0-18.8) and 27.2% (95% CI 18.7-36.5) respectively. Median number of co-morbidities per patient was 1 (range 0-3); median HCT-CI score was 3 (range 0-7). HCT-CI was graded as low (score 0, n=12), intermediate (score 1-2, n=34) or high (score >2, n=50).

**[P645]** Table 1. Early non-hematological toxicity

| | Age group | | |
|---|---|---|---|
| | 18 – 59 | ≥60 | |
| Toxicity | 271 pts., 281 transplants = 100% | 44 pts., 44 transplants =100% | p |
| Oral mucositis | 53 | 50 | 0.64 |
| grade III and IV | 26 | 34 | 0.26 |
| Diarrhea grade III | 47 | 55 | 0.27 |
| Vomiting grade III | 36 | 40 | 0.76 |
| Abdominal pain | 22 | 23 | 0.94 |
| Neutropenic fever | 72 | 59 | 0.09 |
| Septic shock | 1.8 | 2 | >0.1 |
| Pulmonitis or pneumonia | 1 | 2 | ns |
| Skin rash | 3 | 2 | ns |
| Neurologic complication | 0.7 | 0 | ns |
| Metabolic complication | 1 | 2 | ns |

**[P646]**

| Table 1: Demographics and Transplant Procedures | |
|---|---|
| **Sex** | |
| Male | 67 |
| Female | 29 |
| **Median Age (range)** | 50 (16-71) |
| **Diagnosis** | |
| Acute Myeloid Leukaemia (AML) | 43 |
| Acute Lymphoblastic Leukaemia | 12 |
| Myeloma | 12 |
| Non-Hodgkin Lymphoma | 17 |
| Myelodysplasia | 8 |
| Other | 4 |
| **Conditioning** | |
| Myeloablative | |
| TBI (≥12Gy) + chemotherapy | 22 |
| Non TBI | 11 |
| Reduced Intensity | |
| Fludarabine + TBI (2Gy) | 8 |
| Fludarabine + Melphalan + Campath | 40 |
| Fludarabine + Alkylator + ATG | 13 |
| Other | 1 |
| **Stem Cell Source** | |
| Bone Marrow | 12 |
| PBPC | 84 |
| **Donor** | |
| Sibling | 34 |
| Matched Unrelated | 62 |




Of the 126 recorded co-morbidities, most resulted from abnormal liver (n=28) or respiratory (n=68) function tests; no patients had moderate or severe hepatic/lung function by clinical criteria. HCT-CI was non-discriminatory for overall survival (OS, p=0.29) and non relapse mortality (NRM, p=0.88) at 1 year post transplant (Table 1, Figure 1). A similar pattern was observed if analysed by diagnosis or if measured pulmonary/hepatic function were not included in the HCT-CI score. In univariate and multivariate analysis, underlying diagnosis was the most significant determinant of post transplant outcome (Figure 1).
Patients referred for transplantation are highly selected, and typically have little clinical co-morbidity. Conventional co-morbidity indices are of limited value in risk stratification. Inclusion of laboratory assessment of organ function appears to over estimate risk and as applied within the HCT-CI, did not increase the sensitivity of the analysis. In this series, the HCT-CI did not predict outcome following allo-SCT. This may be explained in part by differences in patient demographics and diagnosis compared to those used in the original series and questions its broad applicability.

**P647**

**The influence of ABO incompatibility in transfusion requirements for patients diagnosed with primary immune deficiency treated with allogeneic stem cell transplantation**

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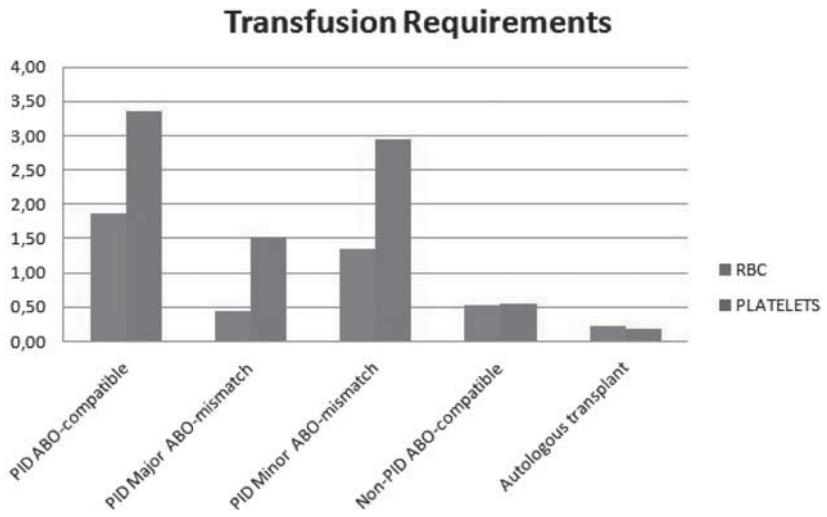
Objectives: To evaluate the influence of different types of ABO Incompatibility in transfusion requirements (TR) during the first 2 months following Allogeneic Stem Cell Transplantation (AST) in patients diagnosed of Primary Immune Deficiency (PID).
Materials and Methods: Medical records of 21 patients aged from 2 months to 7 year-old diagnosed of PID treated with AST were reviewed restrospectively. The AST were conducted in our Centre between July 2001 and July 2011. Data about ABO Incompatibility and TR were collected. TR were studied as the ratio between the number of red blood cells (RBC) and platelets units transfused and the patient´s volemia during the first 2 months following AST.
Results: Stem cell sources were: Bone marrow in 7 patients (33,3%), Peripheral blood in 8 (38,1%) and cord blood in 6 (28,6%). 20 patients received Conditioning treatment and 13 patients received Graft versus Host Disease prophylaxis (the remaining 8 patients received T-cell-depleted AST through CD34+ selection). Nine out of the 21 patients received an ABO-incompatible AST, with major ABO-mismatch in 6 patients (28,6%) and minor ABO-mismatch in 3 patients (14,3%). The remaining 12 AST were ABO-compatible (57,1%). The mean TR were 1,39 (0,69-1,62) for RBC units and 2,78 (0-16,26) for platelets units. Mean TR for RBC units were 0,43 (0-0,76) in the major ABO-mismatch group, 1,35 (0,95-1,99) in minor ABO-mismatch and 1,88 (0,95-5) in ABO-compatible group. Mean TR for platelets units were 1,52 (0-4,28) in major ABO-mismatch, 2,95 (0,48-6,82) in minor ABO-mismatch and 3,36 (0,55-16,26) for ABO-compatible AST. In our Centre, TR for ABO-incompatible-mismatch in non-PID patients were 0,52 for RBC units and 0,55 for platelets units, and TR for Autologous Stem Cell Transplant were 0,23 for RBC units and 0,18 for platelets units.
Conclusion: Patients diagnosed of PID who receive AST present higher TR, independently of age and weight, than non-IPD patients. No clear relation with ABO-incompatibility was found.

[P647]




| TRANSFUSION REQUIREMENTS | | | |
|---|---|---|---|
| Ratio RBC/Platelets/volemia (Liters) | | | |
| | | RBC | Platelets |
| Primary Immune Deficiency | ABO-compatible | 1.86 | 3.36 |
| | Major ABO-mismatch | 0.43 | 1.52 |
| | Minor ABO-mismatch | 1.35 | 2.95 |
| Non-PID ABO-mismatch | | 0.52 | 0.55 |
| Autologous transplant | | 0.23 | 0.18 |

**P648**

**Transplantation outcomes of patients who did not receive methotrexate on day 11 post-transplant because of severe mucositis and life-threatening infection**

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Aim: The disadvantages of a short course of methotrexate for GVHD prevention in allogeneic stem cell transplantation are mucositis and delayed hematologic recovery. We have omitted methotrexate infusion on day 11 posttransplant in patients that had severe mucositis or life-threatening infection after conditioning. The aim of this study is to know if this modification may increase the risk of GVHD and lower the survival of those patients.

Method: We analyzed the transplant outcomes comparatively between patients who received the full schedule of methotrexate (standard group) and patients who did not receive methotrexate on day 11 posttransplant because of severe mucositis or life-threatening infection (modified B). A total of 51 patients were enrolled. Twenty four patients were in the standard group and twenty seven were in the modified group.

Results: Neutrophil recovery and platelet recovery are not different between the two groups (P=0.165 and 0.304, respectively). The number of patients that required anti-CMV treatment (P=0.773) and the number of patients that had CMV disease (P=0.289) did not differ significantly. The cumulative incidence of acute GVHD (grade ≥2) was 25.6% in the standard group and 29.13 % in the modified group (P=0.581). That of chronic GVHD at 2 years was 76.4% in the standard group *vs.* 54.7% in the modified group (P=0.096). Non-relapse mortality (36.7% in standard group *vs.* 36.7% in modified group; P=0.877) and relapse incidence (P=0.892) at 3 years were not different between the two groups. Disease-free survival and overall survival at 3 years did not differ between the two groups.

Conclusion: The transplantation outcomes of patients that did not receive methotrexate on day 11 posttransplant due to severe mucositis or life-threatening infection were not different from those of patients that received the full schedule of methotrexate. This study demonstrates methotrexate infusion on day 11 post-transplant can be omitted without increase of risk of GVHD and non-relapse mortality in transplanted patients who had severe mucositis or life-threatening infection after conditioning.

**P649**

**A review of the transfusion needs in allograft procedures during the last 12 years in bone marrow transplantation unit, Algiers**

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Introduction: Hematopoietic stem cell transplantion (HSCT) patients often require blood and platelet support. Actually, the use of new reduced toxicity regimens conditioning with a shorter duration of aplasia may have an influence on the transfusions needs. In the ABO mismatched transplantation, the transfusion needs seems higher. This study is a review of transfusions needs during 12 years for 1123 procedures of allograft.
Patients and Methods: Between March 1998 to December 2010, 1123 HSCT were performed in 1110 pts, (316 for non-malignancy affection, and 794 for malignant disorders).The median age is 27 years (3-61)and sex-ratio:1,3. The type of conditioning regimen used is: myeloablative (MA) in 783 pts, reduced regimen (RIC) in 209 pts, reduced toxicity conditioning (RTC) in 110 pts. Blood transfusion concerned pts with a hemoglobin rate <7g/dl. For the platelets, the transfusion rate is <10.000/mm3 (pts without bleeding or fever). The stem cell sources were (PBSC:1077, bone marrow:53, cord blood:9). A cryopreservation of PBSC or depletion of erythrocytes in bone marrow were done in 217 cases (19,3%), for major ABO or RH mismatch. The median duration of the aplasia is 14 days (7-86), the median time to achieve HB>7g/dl is 15 days, in the case of major ABO incompatibility the duration of aplasia is longer, the median time to achieve HB>7g/dl is 85 days (7-338).
Results: 2807 blood units (BU) for 711 pts (64%) and 2765 Platelet Concentrates (PC) for 901 pts (81,1%) were necessary, with a transfusion quotient (TQ) of 2,5 units/pt for both blood and platelets. The Blood needs looked higher in the (MA) procedures, 2740 BU with a TQ of 3,5 BU/pt were used versus only 38 BU with a TQ of 0,59 BU/pt in RIC (p=10-8), and just 29 BU, with a TQ of 0,8 BU/pt (p=10-8) in the RTC were used at least. In the particular case of a Major ABO mismatched between donor and recipient, the blood transfusion needs are also higher, the rate is about 6 BU/pt in (MA) regimen (p=10-9) versus 1,1 in RIC (p=10-8) and about 1,42 in RTC (p=10-8). For the use of the PC, in (MA) regimen the needs are higher than in RIC, with 2604 PC used with a TQ of 3,5/pt versus 24 PC with a TQ of 1,1 in RIC (p=10-8), but no differences are noted in platelets needs between (RTC) and (MA) regimen.
Conclusion: The transfusion needs are clearly higher in myeloablatives procedures, they are also very important in the case of Major ABO mismatch. The blood group must be an important criteria for choosing the donor.

**P650**

**A study comparing two types of rabbit ATG as part of pre-transplant conditioning for allogeneic HSCT for haematologic malignancies: earlier relapse with thymoglobulin as compared to ATG-F**

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Aim: To analyze the outcome of two different rabbit polyclonal antilymphocyte globulins in allogeneic hematopoietic stem cell transplantation (alloHSCT) after reduced intensity conditioning (RIC) for hematological malignancies.
Methods: In order to avoid cost containment measures we alternated between the 2 ATG sources (Thymoglobulin and ATG-F) in patients with hematologic malignancies receiving a reduced intensity conditioning regimen. In this single-center retrospective analysis, we included 30 alloHSC recipients transplanted between 2007 and 2010, 15 patients received Thymoglobulin (2.5 mg/kg/day from day -5 to day -3 before transplant) and 15 received ATG-F (5 mg/kg/day from day -6 to day -2 before transplant). In addition the conditioning regimen consisted of busulphan and fludarabine. We compared the incidence of adverse effects, graft failure, relapse, infection, graft versus host disease (GvHD), immune reconstitution and death.
Results: The two groups were homogeneous. Median follow up was 29 (11-49) months. Out of the 30 patients included, 15 in each group, a significantly earlier relapse is noticed in the Thymoglobulin group (median time to relapse 91 days) compared to the ATG-F group (325 days) (p=0.01). This was not related with T-depletion neither with the number of cells in the add back of T cells. The number of relapse was similar in both groups. There was a tendency to show more adverse events during the Thymoglobulin perfusion than with that of ATG-F (p=0.14), less acute graft versus host disease grade II-IV (p=0.23) and an

**[P650]**

| Complications | A n = 15 | T n = 15 | P |
|---|---|---|---|
| Adverse effects of antilymphocytic globulin, n (%) | 4 (27) | 8 (53) | 0.14 |
| Rejection, n (%) | 2 (13) | 3 (20) | 1.00 |
| Time to rejection, median (range, days) | 48 (39-57) | 39 (28-72) | 0.93 |
| Relapse, n (%) | 4 (27) | 7 (47) | 0.44 |
| Time to relapse, median (range, days) | 325 (102-412) | 91 (14-183) | 0.01 |
| CMV reactivation, n (%) | 11 (73) | 10 (67) | 0.69 |
| Severe infection after aplasia, n patients (%) | 5 (33) | 6 (40) | 0.71 |
| Acute GvHD grade 0-I, n (%) | 2 (13) | 2 (13) | 1.00 |
| Acute GvHD grade II-IV, n (%) | 6 (40) | 3 (20) | 0.23 |
| Chronic limited GvHD, n (%) | 0 | 0 | 1.00 |
| Chronic extensive GvHD, n (%) | 4 (27) | 3 (20) | 0.67 |
| Death, n (%) | 7 (46) | 5 (33) | 0.46 |
| Time to death, median (range, months) | 10 (3-16) | 5 (3-8) | 0.07 |

A=ATG-F, T=Thymoglobulin, GvHD = graft versus host disease

earlier death in the Thymoglobulin group (p=0.07). Engraftment was similar in both group, as well as rejection, infections after aplasia, CMV reactivation, secondary neoplasm and immune reconstitution. The OS, relapse incidence and TRM at 2 years were 60±18%, 42±20% and 26±16% respectively for the entire population and there was no statistical difference between both groups.
Conclusion: In patients with hematologic malignancies, conditioning with Thymoglobulin seems to be associated with a significantly shorter time to relapse compared to the ATG-F group, and a tendency to die earlier and to present more perfusion adverse events, but less grade II-IV GVHD. The immunosuppressive power of Thymoglobulin seems to be greater than that of ATG-F.

**P651**

**Caphosol in prevention of oral mucositis in autologous stem cell transplant recipients after high-dose melphalan (CASH)**

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Background: Caphosol (a supersaturated calcium phosphate rinse) for oral care has been shown to decrease the incidence, severity and duration of oral mucositis (OM) caused by chemotherapy. (OM) leads to a higher incidence of fever, infections, opioid and antibiotic use and prolonged hospital stay. In the prospective OM audit (POMA study) autologous haematopoietic stem cell transplant (ASCT) recipients received 200 mg/M² of melphalan (HDM) (1). Recalculation of the given dose per kilogram bodyweight was the key factor determining OM incidence and severity. All patients that received ≥ 5.25 mg/kg melphalan besides standard oral care (OC) developed OM with 65% severe OM (WHO grade 3-4).
Aim: To evaluate the prevention of severe OM with Caphosol in ASCT recipients receiving ≥ 5.25 mg/kg Melphalan.
Method: A multicenter, observational prospective cohort audit was performed in 154 ASCT recipients treated with HDM (200 mg/M²) for multiple myeloma. Patients with melphalan ≥5.25 mg/kg after recalculation received standard OC and Caphosol 4 times daily. Patients with < 5.25 mg/kg melphalan received standard OC only. OM was assessed daily using the WHO oral toxicity scale (0-4) from day 1 of starting HDM in the first 20 days afterwards. Neutropenic fever (defined as temperature > 38°C and a neutrophil count of < 0.5 109/L), time to neutrophil engraftment, duration of hospitalisation, concomitant medications and incidence of infections were also monitored.
Results: 47 patients received actually ≥ 5.25 mg/kg melphalan and were ordered Caphosol treatment. The maximum mean WHO OM grade was not significantly different of the group with standard OC only. The incidence of severe OM in the Caphosol group was only 45% in stead of expected incidence of 65% (POMA). Neutropenic fever occured in 25 patients in the Caphosol group (53%) and in 81 patients (76%) in the group with standard OC (p=0.0055).
Conclusion: Caphosol is effective in prevention of (severe) OM and neutropenic fever in ASCT recipients after HDM.
(1) Blijlevens N, Schwenkglenks M, *et al.* Prospective oral mucositis audit: oral mucositis in patients receiving high-dose melphalan or BEAM conditioning chemotherapy. J Clin Oncol 2008 (26): 1519-1525.

**P652**

**Recombinant human soluble thrombomodulin is effective in the treatment of early complications after haematopoietic stem cell transplantation in children and adolescents**

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Background: Early complications of vascular origin including hepatic SOS, TMA after HSCT remain serious problems. Recombinant human soluble thrombomodulin (rTM) has been shown to be effective in the treatment of DIC. Recently successful treatment of hepatic SOS has been reported although in a small study. We report here results of retrospective analysis in a group of 67 patients (pts) who received rTM after HSCT.
Patients and Methods: Enrolled in this study were children and adolescents with autologous and allogeneic HSCT who were treated with rTM. The data were collected retrospectively from 30 unselected Japanese centers that joined in the JPLSG. Pts were divided into 4 cohorts based on the aim of rTM, treatment of DIC (group 1), treatment of SOS (group 2), treatment of TMA (group 3), pre-emptive or prophylactic treatment of SOS/TMA (group 4). When indicated, rTM was given as a single daily infusion at a dose of 380 U/kg. Complete response (CR) was defined as resolution of each complication-related symptoms with the related laboratory findings decrease to less than diagnostic range. Treatment failure was defined as either unable to obtain CR in treatment or newly development of each complication in pre-emptive or prophylactic treatment.
Results: The median age at HSCT was 6.4 years (range, 0.6 to 18 years), 39 were male and 28 were female. The underlying diseases were ALL (22), AML (16), other malignancies (21), and nonmalignant disease (8). Thirty-six pts were in early stage (1CR or 2CR), 23 pts were in advanced stage (3CR or more). The donor was HLA-identical sibling in 7, other related in 15, unrelated BM in 14, unrelated CB in 20, autologous in 10, unknown in 1, respectively. Once daily administration of rTM was started between day +1 and day +96 (median day +10). Fifteen pts received rTM for the treatment of DIC, 21 pts for SOS, 16 pts for TMA, and 15 pts for pre-emptive treatment or prophylaxis. CR rate was 73% (11/15 pts) in group 1, 90% (19/21 pts) in group 2, 69% (11/16 pts) in group 3, respectively. Survival rate was 67% (10/15 pts) in group 1, 71% (19/21 pts), 63% (10/16 pts) in group 3, respectively. In group 4, 2 pts developed TMA, and 67% (10/15 pts) are surviving. Adverse event was recognized in 3 TMA pts.
Conclusions: Although this study is a retrospective analysis about limited number of pts, rTM may be highly effective in early complications of vascular origin after HSCT, especially in hepatic SOS.

**P653**

**Romiplostim for post-transplantation failure of platelet recovery**

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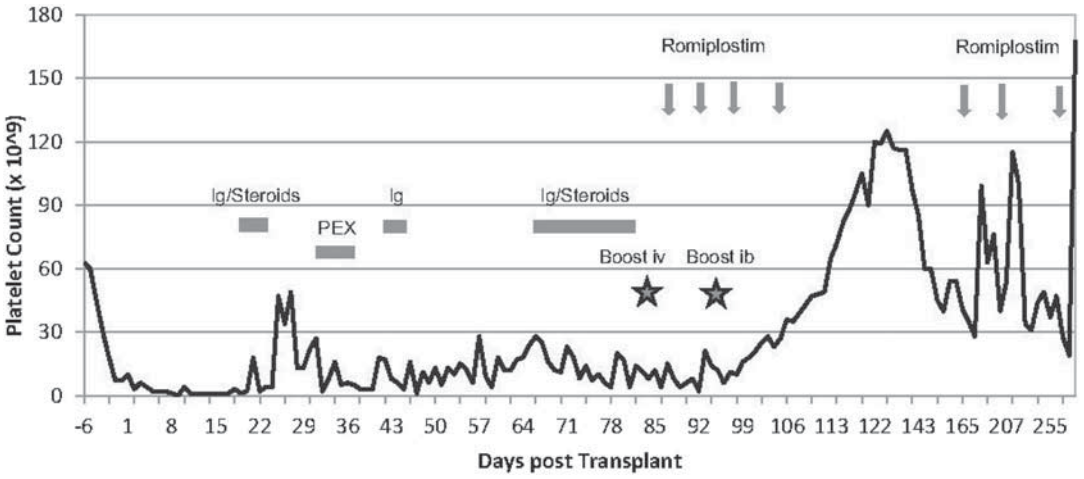
Thrombocytopenia following chemotherapy or stem cell transplantation (SCT) results in increased used of blood products and hospital resources, reduced quality of life and in general increased morbidity and mortality. Romiplostim (Rom) is a thrombopoietin analogue indicated for chronic immune thrombocytopenic purpura as second line treatment in adults failing or not eligible to splenectomy. We report 3 cases of successful off-label use of Rom for chemotherapy or SCT related thrombocytopenia. A 68 years old female had unexplained failure of platelet (plt) recovery following autologous SCT ($6.5x10^6$CD34/kg) for acute

[P653]




myeloid leukemia. Despite receiving multiple platelet transfusions, her plt count remained <10x10$^9$/L (Figure). Autoimmune thrombocytopenia was suspected, supported by the concomitant development of Guillen Barrè syndrome. Standard therapy was ineffective including steroids, immunoglobulins (Ig), plasmaexchange (PEX), intravenous (iv) and intrabone (ib) autologous boosts. Because severe thrombocytopenia was limiting physiotherapy and therefore neurologic recovery, Rom was started at 1 mcg/kg escalated to 4 mcg/kg for a total of 4 weekly subcutaneous doses. Nine days after the last administration plt rose to 100x10$^9$/L. Discontinuation resulted in reduction of plt count but further 0.5 mcg/kg monthly maintenance injections were successful in keeping plt >30x10$^9$/L. A 68 years old male underwent unrelated SCT for high risk acute lymphoblastic leukemia. Primary failure of platelet recovery occurred following hyperacute, acute and overlap chronic GVHD. Because of an important muco-cutanous hemorrhagic diathesis, Rom was initiated at 2 mcg/kg weekly. Injections were stopped after 2 months as plt counts reached 47x10$^9$/L. Two months from Rom completion, plt continue to rise and are currently 100x10$^9$/L. A 65 years old male affected by relapsed multiple myeloma was candidate to lenalidomide treatment but, in spite of disease response, he was unable to continue because of dose limiting thrombocytopenia. Rom was started at escalating doses of 1 mcg/kg to 4 mcg/kg. When plt reached 100x10$^9$/L Rom was stopped. One month later, whilst continuing lenalidomide, plt dropped again but further injections were successful in allowing continuation of lenalidomide courses. In conclusion, in selected cases, Rom could be cautiously used to treat chemotherapy or transplant related thrombocytopenia.

**P654**

**Haploidentical stem cell transplantation as immediate rescue from primary graft failure**

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Early identification of primary graft failure is essential to guide rescue strategies due to high mortality of prolonged aplasia. Two recent cases of primary graft failure received salvage T replete haploidentical stem cell transplantation (haplo-SCT) at our Institution.

A 62-year-old CMV+0+male received a volunteer unrelated donor (VUD) SCT for idiopathic JAK-2neg myelofibrosis with splenomegaly and transfusion related hemocromatosis (Figure). Conditioning: Busulfan 12 mg/kg, Fludarabine (Flu) 160 mg/mq, ATG-thymo 5 mg/kg. Stem cell source: 5.3x10$^6$ CD34/kg peripheral blood stem cells (PBSCs) from a CMV-AB+ male, 10/10 HLA matched. Day 28 bone marrow (BM) showed aplasia with 0% donor chimerism (CMV/HSV6/parvo neg). The haplo 32 years old CMV- B- son underwent urgent mobilization. Conditioning: treosulfan (treo) 42 g/mq, Flu 150 mg/mq, ATG-F 30 mg/kg and Rituximab 500 mg. PBSCs: 8.02x10$^6$/kg CD34+/kg, 2.1x10$^8$ CD3/kg. GvHD prophylaxis: MTX and rapamycin. Post transplant complications: BKvirus hemorrhagic cystitis and HHV6 viremia. Day 55 BM showed 100% donor chimerism. Because of poor graft function on day 83 an intra-bone boost of 1.37x10$^6$/kg CD34+ donor PBSCs was given. Engraftment: neutrophil on day 70 post boost, plt on day 8. At 13 months follow-up the patient is in remission, with moderate de novo chronic GVHD. A 56-year-old pluriparous CMV+A+ female received a VUD SCT as upfront treatment for chronic myelomonocytic leukemia. Stem cell source: 8.3x10$^6$ CD34/kg PBSCs from a CMV+A+ male, mismatched at HLA-B and DPB1.

Conditioning: Thiotepa 10 mg/kg, treo 42 g/mq, Flu 150 mg/mq, ATG-F 30 mg/kg and Rituximab 500 mg. Day 22 BM showed aplasia with 0% donor chimerism (CMV/HSV6/parvo neg). The haplo 18 yrs old CMV- A + son underwent urgent mobilization. Conditioning: Flu120mg/mq, ATG-thymo 15 mg/kg, Rituximab 600mg and TBI 2Gy. PBSC: 7.02 x10$^6$ CD34+/kg, 0.87x10$^8$ CD3/kg. GvHD prophylaxis: MTX and rapamycin. Engraftment: neutrophil >500/mcL on day 20 and plt >20,000/mcL on day 40. Post transplant complications: grade III febrile neutropenia, interstitial pulmonitis, acute pulmonary edema and CMV viremia. Day 30 BM showed 100% donor chimerism. At a 4 months follow-up the patient is in remission, without GVHD and off immunosoppression.

In conclusion, availability of a haplo-donor could be regarded as an alternative to autologous back-up as rescue strategy from primary graft failure in patients undergoing VUD SCT for high risk haematological malignancies.

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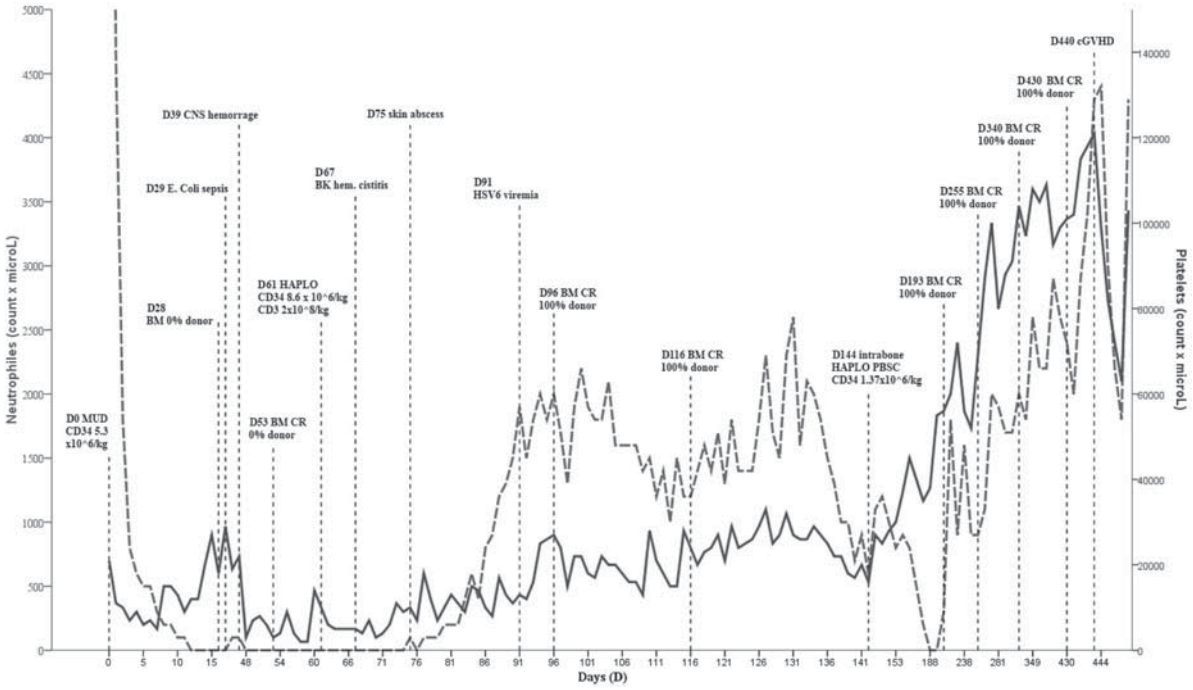


**P655**

**Association of aprepitant, palonosetron with or without dexamethasone in the prevention of nausea and vomiting in patients treated with high-dose chemotherapy and autologous bone marrow transplantation**

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Introduction: Complete protection from nausea/vomiting is currently achieved in a small number of patients (pts) receiving high-dose chemotherapy (HDC). Currently the use of 5-HT3-antagonists and dexamethasone (Dexa) represent the standard of care. The role of the NK-1-antagonist aprepitant (Aprep) in HDC remains to be better defined; however, recently have been reported good responses with triple antiemetic combination (5-HT3-antagonists, Dexa and Aprep). This study describes the addition of Aprep to palonosetron with or without Dexa with the aim of preventing nausea and vomiting associated with HDC in autologous stem cell transplantation (ASCT) patients.
Patients: We treated 14 pts (5 Non Hodgkin Lymphoma, 1 Hodgkin disease and 8 Multiple Myeloma (MM)). The pts with lymphoma underwent to HDC with BEAM (carmustine 300 mg/m$^2$ on day -7, etoposide 200 mg/m$^2$ and cytarabine 400 mg/m$^2$ on days -6, -5, -4, -3 and melphalan 140 mg/m$^2$ on day -2) and the pts with MM with Melphalan 200 mg/m$^2$ on day -1. The Aprep was used p.o. at a dose of 125 mg on day -7 and 80 mg on days -6, -5 in pts treated with BEAM and at a dose of 125 mg on day -1 and 80 mg on days 0 and +1 in pts treated with M200; the palonosetron was used at a dose of 250 µg on days -7 and -4 nei pts treated with BEAM and on day -1 nei pts treated with MEL200; the Dexa has been used only in pts treated with BEAM at a dose of 12 mg e.v. on day -7 and 8 mg e.v. in the remaining days of HDC.
Results: Complete remission was defined as no emesis and no use of rescue therapy during day of HDC (acute phase) and until 5 days after the end of HDC (delayed phase). For the overall evaluation phase the primary end point of CR was achieved in 5 (36%) patients. For the acute phase, CR was achieved in 10 pts (71,4%). Delayed nausea (grade 1 according to WHO) was observed in 7 pts (50%) and delayed vomiting in 2 pts (14,3%).
Conclusion: In our study Aprep has demonstrated good tolerability. Despite of the experience in a small subset of the pts, we believe that the combination of Aprep and palonosetron with or without Dexa show a good response in the acute phase (71,4%) and the sharp reduction in episodes of vomiting. This association appears to control less well the delayed nausea whereby the pts treated with BEAM could benefit from an extension of the Aprep with 80 mg from until the end of HDC. The pts treated with M200 could benefit from the addition of Dexa (8 mg daily on days -1, 0, +1).

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**Romiplostim (AMG531, Nplate®) for secondary failure of platelet recovery after allogeneic stem cell transplantation**

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Romiplostim (Rm), a protein that binds to and stimulates the thrombopoietin receptor, has been shown to increase and maintain platelet counts in patients with chronic immune thrombocytopenic purpura (ITP). Thrombocytopenia after allogeneic stem cell transplantation (allo-SCT) is a common complication, but some patients present with secondary failure of platelet recovery (SFPR) defined as a decline of platelet counts below 20,000/µL for 7 consecutive days or requiring transfusion support after achieving sustained platelet counts ≥50,000/µL for 7 consecutive days after allo-SCT. We report on seven patients treated with Rm after allo-SCT for a SFPR not due to disease relapse. Our therapeutic strategy was to start Rm at 1µg/kg and to increase dose weekly until platelet count had reached 50,000/µL.
Transplantations were performed between 2009 and 2011. The median age of the patients was 57 years (range, 25 to 60). Diseases were distributed as follow: acute myeloid (n=2) or lymphoid (n=1) leukemia, myelodysplastic syndrome (n=1), non-Hodgkin lymphoma (n=2), severe aplastic anemia (n=1). At the onset of SFPR, all patients had severe thrombocytopenia (<10,000/µL). The median time between allo-SCT and onset of SFPR was 3 months (range, 2 to 18). The median time between

onset of SFPR and start of Rm was 27 days (range, 7 to 61). Thrombocytopenia was corrected in all patients. There was a median time of 54 days (range, 24 to 84) between onset of Rm and the first day when platelet count was >50,000/μL. Median duration of treatment was 13 weeks (range, 4 to 16). Rm was well tolerated and no patient needed to discontinue drug. Four patients developed SFPR within 2 months of developing acute GvHD II-IV. Two patients had an acute GvHD II-IV before and had 2 episodes of CMV or EBV infection. The last patient did not develop GvHD II-IV but had a CMV infection 1 month before SFPR. These observations underline the occurrence of SFPR shortly after episodes of acute GvHD II-IV, BOOP, EBV or CMV infections. At the last follow-up, one patient had died of a relapse of acute myeloid leukemia while others were alive, in remission without recurrence of SFPR after discontinuation of Rm.
We conclude that Rm can be a therapeutic option after allo-SCT for patients with severe thrombocytopenia caused by SFPR. However, these preliminary data need to be confirmed in larger studies with a longer follow-up time to improve assessment of efficacy and safety profiles within this setting.

**P657**

**Romiplostim in treatment of thrombocytopenia after allogeneic haematopoietic stem cell transplantation**

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Objectives: To evaluate the effectiveness of trombopoetin receptor agonist romiplostim in patients (pts) after allogeneic hematopoietic stem cell transplantation (allo-HSCT) who developed severe refractory thrombocytopenia due to posttransplant complications.
Methods: To date 3 male pts with acute leukemia with median age 22,3 (18-30) years were included in the study. At the time of allo-HSCT one pt was in chemorefractory relapse and two pts in second complete remission (CR). After reduced intensity conditioning two pts received haploidentical bone marrow and peripheral blood stem cells (PBSC) and 1 pt received matched unrelated donor PBSC transplantation.
Engraftment was achieved in the median of 17 days (12-21), full donor chimerism and CR were observed during follow-up period. Reconstitution of platelet count: ≥20x10$^9$/l in the median of 14,6 days (12-19), ≥100x10$^9$/l in the median of 24,3 days (21-27).
All pts developed thrombocytopenia due to persistent cytomegaloviral (CMV) infection, acute graft versus host disease (aGVHD) and thrombotic thrombocytopenic purpura (TTP) in the median of 64,6 days after allo-HSCT (range 38–80 days). All of them were treated with methylprednisolone 1mg/kg, gancyclovir 5-10 mg/kg, immunoglobulin 0,5 g/kg (2 pts), rituximab 375 mg/kg (1 pt) but remained profound thrombocytopenic (5-10X10$^9$/l) and transfusion dependent with severe hemorrhagic complications in two cases.
Romiplostim was administered in the initial dose 1 mkg/kg weekly with dose escalation to 4 mkg/kg (2 pts) and 5 mkg/kg (1 pt). Treatment period was 5 weeks (2 pts) and 8 week (1 pt).
Results: In two weeks all pts achieved stabilization of platelet count ≥ 15x10$^9$/l with transfusion independency and completion of bleeding. After 5 weeks of treatment platelet count was more than 30x10$^9$/l, 50x10$^9$/l and 150x10$^9$/l accordingly. No romiplostim associated toxicity and recurrence of thrombocytopenia was observed during follow-up period.
Conclusions: Using of romiplostim in pts after allo-HSCT who developed refractory thrombocytopenia due to TTP, CMV infection and aGVHD seems to be effective and well tolerated. The study is ongoing.

**P658**

**Oral cryotherapy is useful and safe in the prevention of oral mucositis after conditioning regimens with high-dose melphalan for lymphoma and myeloma**

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Background. Oral mucositis (OM) is a common complication of conditioning regimens with high dose melphalan (HDmel) such

**[P658]**

| | NO OC (n=76) | OC (n=74) | p-value |
|---|---|---|---|
| OM | 62 (82%) | 32 (43%) | < 0.001 |
| OM grades III-IV | 21 (28%) | 10 (14%) | 0.033 |
| Onset of OM after mel (days), mean (SD) | 2.50 (1.02) | 2.97 (1.31) | 0.129 |
| Duration (days) OM, mean (SD) | 9.44 (2.41) | 8.75 (2.49) | 0.297 |
| Infection | 70 (92%) | 61 (82%) | 0.075 |
| Days on antibiotics, mean (SD) | 10.80 (5.14) | 9.30 (4.59) | 0.050 |
| Parenteral nutrition | 13 (17%) | 11 (15%) | 0.708 |
| Narcotics | 15 (20%) | 10 (14%) | 0.307 |
| Days hospitalization, mean (SD) | 23.45 (5.85) | 22.32 (5.49) | 0.209 |

as BEAM for lymphomas, HDmel in autologous HSCT for multiple myeloma (MM) and fludarabine-melphalan in RIC-HSCT for lymphoproliferative syndromes. This retrospective cohort study analyses the impact of oral cryotherapy (OC) or room temperature saline rinses in the prevention of OM in lymphoma and MM patients submitted to HSCT in a single center.
Patients and Methods: From August 2006 to July 2011 150 consecutive patients were enrolled. Two consecutive groups were included: No OC (August 2006 to April 2009, 76 patients) and OC (May 2009 to July 2011, 74 cases). Median age: No OC 53 yr (range 25-70) *vs* 56 yr (range 23-69) for OC. Gender: 42 males (no OC) *vs* 55 (OC). All MM cases (78, 52%) received HDmel as conditioning regimen (38 no OC, 40 OC), 56 patients (37%) with lymphoma received BEAM (30 no OC, 26 OC) and 16 (11%)(6 CLL, 8 follicular lymphoma) received RIC-HSCT (8 no OC, 8 OC). OC started 5 min before HDmel administration and finished 15 in after HDmel infusion. OM was assessed using the WHO grading scale. OM evaluation began the day after melphalan infusion. Additional data collected included administration of parenteral narcotics and/or nutrition, infection and days of antibiotics and hospitalization.
Results: OM was observed in 94/150 cases (63%): 47/56 (84%) in patients treated with BEAM (grade III-IV in 20/47 [42%]), 38/78 (49%) in patients receiving HDmel (grade III-IV in 11/38 [29%]) and 9/16 (56%) in patients treated with Flu-Mel (grade III-IV in 0/9 [0%]). The frequency and severety of mucositis were significantly higher in patients who received BEAM compared with the remaining (p<0.001). Table 1 summarizes the main outcomes analyzed in the no OC and OC groups.
Conclusions: OM was more frequent and severe in patients treated with BEAM than with HDmel or Flu-Mel. OC reduced the frequency and severity of OM, and a trend for less infections was observed in OC patients. OC did not influence on the beginning and duration of OM, parenteral nutrition and narcotics, as well as on days of antibiotics or hospitalization.
Supported by grant P-EF from FIJC and RD06//0020/1056 from RTICC, Instituto Carlos III, Spain.

**P659**

## At home autologous stem cell transplantation in older patients with lymphoma and multiple myeloma. A single-centre experience

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Aim: To analyse the clinical outcome of home care autologous stem cell transplantation (ASCT) in patients older than 60 years diagnosed with lymphoma or multiple myeloma (MM).
Patients and Methods: At home ASCT (since day +1) was offered to patients with a good performance status, a travelling time to the hospital < 60 minutes, and a caregiver available 24h a day. The preparative regimen was administered in the hospital: BEAM for patients with lymphoma and melphalan 200 (MEL200) for patients with MM. All patients received prophylactic i.v. ceftriaxone once daily. The nurse visited the patient at home once or twice daily, while the physician in the hospital visited the patient only in case of clinical complications. Indications for re-admission to the hospital were: willingness of the patient or caregiver; uncontrolled nausea, vomiting or diarrhoea; mucositis requiring total parenteral nutrition or i.v. morphics; fever with focal infection or signs of severe sepsis.
Results: 107 patients were included (73 received BEAM and 34 MEL200), with a median (range) age of 47 (17-67) years. Twenty-two (20%) of them were older than 60 years, median (range) of 64 (60-67), 11 BEAM and 11 MEL200. Main clinical outcomes are shown in the table. Whereas in the MEL200 group the age did not influence the degree of toxicity after ASCT, older patients receiving BEAM showed more prominent toxicity than younger patients treated with the same conditioning regimen. Thus, older lymphoma patients showed higher grade II-IV intestinal toxicity (64% *vs.* 14%, p=0.001), required two daily visits by nurses more frequently (82% *vs.* 45%, p=0.05), a more frequent need of physician visits (p=0.004), and more frequent readmission (45% *vs.* 13%, p=0.03). Besides, older lymphoma

**[P659]**

| | BEAM | | p value | Melphalan 200 | | p value |
|---|---|---|---|---|---|---|
| | < 60 years (n=62) | >=60 years (n=11) | | < 60 years (n=23) | >=60 years (n=11) | |
| **Age in years, median (range)** | 42 (17-58) | 61 (60-64) | <0.0001 | 47 (25-58) | 64 (61-67) | <0.0001 |
| **CD34+ cell dose (x10^6/kg)** | 3.4 (1.5-6.8) | 2.7 (2.4-10.3) | NS | 3.6 (1.9-21.6) | 2.7 (2.4-10.3) | NS |
| **Time to engraftment** | 11 (9-26) | 10 (9-14) | NS | 11 (10-26) | 12 (10-18) | NS |
| **Incidence of fever** | 82% | 91% | NS | 52% | 55% | NS |
| **Grade II-IV mucositis** | 35% | 36% | NS | 4% | 0% | NS |
| **Grade II-IV intestinal toxicity** | 14% | 64% | 0.001 | 4% | 9% | NS |
| **Two daily visits by nurses** | 45% | 82% | 0.05 | 13% | 18% | NS |
| **Physician visits** | 2 (1-5) | 3 (1-6) | 0.004 | 1 (1-5) | 2 (0-3) | NS |
| **Readmission** | 13% | 45% | 0.03 | 9% | 18% | NS |

patients had higher grade II-IV mucositis (36% *vs.* 0%, p=0.02) and intestinal toxicity (64% *vs.* 9%, p=0.02), required two daily visits by nurses more frequently (p=0.009), and more frequent physician visits (p=0.03), than older MM patients.
Conclusion: At home ASCT program in older patients is feasible and safe. However, BEAM regimen for lymphoma was associated in the older group with more severe mucositis and intestinal toxicity, and increased need for home care and hospitalization. In contrast, older MM patients tolerated well MEL200.

**P660**
**Impact of induction intensity in multiple myeloma patients on autologous haematopoietic cell transplantation morbidity – a single-centre experience**
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Background: In pts with multiple myeloma who are eligible for autologous hematopoietic cell transplantation (HCT), induction therapy has evolved from chemotherapy-based therapy to novel agents-based regimens.
Objectives: We aimed to compare HCT-associated toxicities in patients receiving combined chemotherapy and novel agents-based treatment compared to those receiving novel agents only-based therapy.
Methods: We retrospectively reviewed all charts of patients with multiple myeloma who were given induction therapy and HCT. Patients given combined regimen (dexamethasone, thalidomide, bortezomib cisplatinum, adriamycin, cyclophosphamide and etoposide, DTPACE) were defined as DTPACE group. Patients given only novel agent-based regimens were defined as non-DTPACE group.
Results: Between the years 2005 and 2011, 38 pts received DTPACE-based regimen and 31 pts were given non-DTPACE-based regimen prior to HCT. There was no statistically significant difference between the two groups in patients' age, sex, percentage of patients with ISS>1, comorbidity index score (HCT-CI)>1 and disease status at HCT. Patients given DTPACE had a prolonged neutropenic period (mean, 7 *vs.* 6 days, respectively p=.0002) despite a higher mean CD34 cell transfused dose ($6.5x10^6$ *vs.* $4.7x10^6$, p=.06, respectively). Eight patients (12%) developed clinically documented infections and 11 patients (16%) developed microbiology documented infection (Gram negative bacteremia, n=5, Gram positive bacteremia, n=3, PCP infection, n=3). There was no difference in the incidence of fever or documented (clinically or microbiologic) infections and in blood or platelets transfusion rate between the two group. There was a trend for higher percentage of patients consuming morphine for severe mucositis in patients given DTPACE (35% *vs.* 13%, p=.07). One transplantation associated death (1.4%) was documented in the DTPACE arm. By landmark analysis the median time to next treatment was 38 months in the DTPACE group and was not reached in the non-DTPACE group (HR 0.6, 95% CI 0.16-2.2, p=.3, Figure). At 3-years post HCT, there was no difference in overall survival between the two groups (HR 0.33, 95% CI 0.08-1.4, p=.2).
Conclusions: Higher intensity induction chemotherapy has only a modest impact on HCT-associated morbidity. Longer follow up is warranted to determine its impact on time to next treatment and overall survival.

**P661**
**Outcome of salvage allogeneic haematopoietic cell transplantation in patients failing primary allogeneic transplantation: a single-centre experience**
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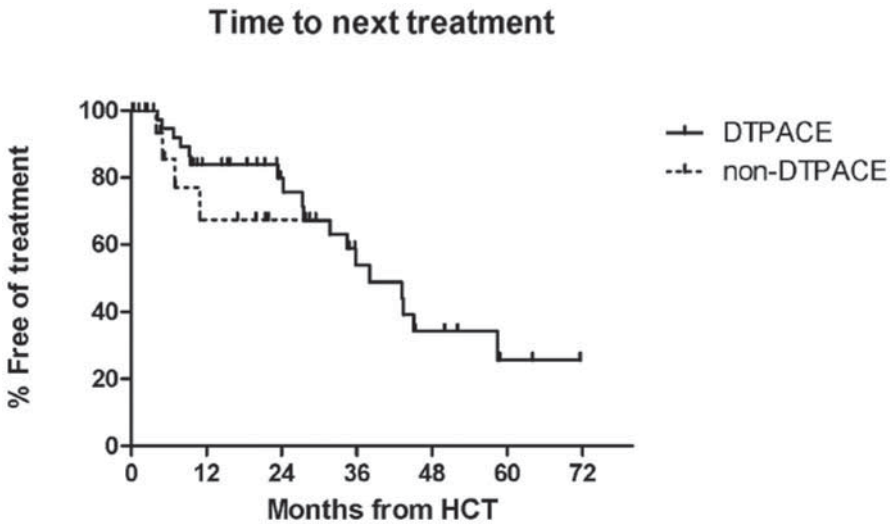
The prognosis in patients with relapse after allogeneic hematopoietic cell transplantation (HCT) is dismal. Salvage allogeneic HCT is one possible treatment option besides palliative care or donor lymphocyte infusions with or without chemotherapy. We retrospectively analyzed our experience with salvage HCT from 2000-2011 to further define patients groups potentially benefitting most from such approach. 47 patients (f=20, m=27) received more than 1 allogeneic HCT (2. HCT=44, 3. HCT=3). Median age of the patients was 40 (range, 18-65) years. Diagnoses were acute myeloid leukemia (n=28), acute lymphoblastic leukemia (n=11), chronic myeloid leukemia (n=2), myelodysplastic syndrome (n=2), osteomyelofibrosis (n=2), multiple myeloma (n=1), and non-Hodgkin lymphoma (n=1). Reasons for salvage HCT were relapse (n=49) or primary graft failure (n=1). Median time between 1. and 2. HCT was 17 months (range, 3-137), between 2. and 3. HCT 12 months (range, 10-16). For the 2. HCT 16 patients received myeloablative conditioning (MAC) and 31 reduced intensity conditioning (RIC). For the 3. HCT RIC was used in all patients. In 8 patients the same donor as for primary HCT was used while in 39 patients an alternative donor was chosen. Before salvage HCT 14 patients were in complete remission (CR), 33 in partial remission (PR). 12 of 47 patients (26%) after 2. HCT are alive (CR=11, PR=1) and 1 of 3 patients after 3. HCT is alive and in CR.

**[P660]**



Kaplan-Meier estimated 3-year overall survival (OS) is 30%, with a median follow-up of 62 (range 6-111) months. Outcome was better for patients in CR at HCT (3-year OS with 43% *vs.* 24%, p=0.13). Older age had no negative impact on survival as 3-year OS in patients 40 years was 43% compared to 15% in patients <40 years (p=0.04). Cumulative incidence of non-relapse mortality at 3 years after 2. HCT was 43%. The use of RIC was associated with an inferior 3-year OS compared to MAC (18% *vs.* 53%, p=0.08). Outcome was inferior if the 2. HCT was within 6 months after 1. HCT (3-year OS of 0% *vs.* 31%, p=0.02). Incidence of grade II-IV graft-versus host disease (GVHD) was 17%, of chronic GVHD 30%. cGVHD after 2. allo HCT was associated with better survival (3-year OS 42% *vs.* 26%, p=0.31) especially after RIC (33% *vs.* 11%, p=0.09).
Salvage HCT after failing primary HCT is feasible and can achieve long term disease free survival in up to a third of patients. It appears to be the most promising salvage strategy besides DLI for relapse >6 months after HCT.

## Aplastic Anaemia

**P662**
**Second haematopoietic stem cell transplantation from unrelated donor in severe aplastic anemia. A retrospective analysis from the Aplastic Anaemia Working Party (SAA-WP)**
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Background: Primary or secondary graft failure after allogeneic hematopoietic stem cell transplantation (HSCT) is still a major problem in patients affected by SAA, and a second allograft is often a needed alternative. We analyzed retrospectively the outcome of the second HSCTs recorded in the EBMT Registry from 1 January 1998 to 31 December 2009.
Patients and Methods: 63 SAA-patients who underwent a second unrelated HSCT for primary or secondary graft failure were the case-patients (pts). 120 SAA-pts who received a second related matched HSCT in the same period were the control group.
Results: Related and unrelated HSCT group presented the following characteristics: median age at second HSCT 21.9 y (range 1.5-64.6) *vs* 20.3 y (range 4.4-56.3); myeloablative conditioning regimen: 23 pts (36,5%) *vs* 55 pts (45.8%); RIC conditioning regimen: 26 pts (41.3%) *vs* 44 pts (36.7%); TBI-conditioning regimen: 22% pts *vs* 17.5% pts. Stem cell source was peripheral blood in 38 pts (60%), bone marrow in 15 pts (24%) and cord blood in 10 pts (16%). Neutrophils engraftment was achieved in 42/63 (67%) of case-pts and in 88/120 (73%) of control-pts. Graft failure/rejection was 25% in the case-group and 28% in the control cohort. Grade I-II, grade III-IV acute GVHD and chronic GVHD were 35%, 6% and 21% in case-group *vs* 20%, 7.5%, and 17.5% in control-group. The main causes of death were infections and GVHD. Overall TRM and 100 day-TRM were 32% and 24% *vs* 29% and 14%, respectively. After a median follow-up of 2.9 y (range 0.2-9.5) for case-group and of 3.5 y (range 0.2-10.6) for control-group, no difference in OS was found: 50% *vs* 61% (p=.3). OS was similar for related and matched unrelated donor (62.1% *vs* 60.8%), whereas a lower OS was found with a mismatched unrelated donor: 35%, H.R. 2.21 (CI 95%, 1.21-4.03). In univariate analysis, a longer interval from first to second transplant (> the median time of 119 days) and the use of myeloablative regimen were associated to a better outcome, OS being 74% and 67% *vs* 39% and 50%, p<.01. In the subgroup of unrelated HSCT, OS was influenced by HLA match (matched *vs* mismatched: 60.8 *vs* 34.6, p=.017) and by the intensity of conditioning regimen (myeloablative *vs* RIC: 78.2% *vs* 16.4% (p=.0013).
Conclusion: second HSCT in SAA-pts has a comparable outcome both with an unrelated and a related donor. The factors associated to OS were HLA match, intensity of conditioning regimen and interval between 1st and 2nd transplant.

**P663**
**The incidence of autologous recovery and late graft failure in a Brazilian cohort of SAA transplanted patients**
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Two types of graft rejection can occur following allo-BMT: primary graft failure (PGF) where there is no evidence of recovery of donor cells beyond D+21, and secondary graft failure (SGF) associated with loss of donor chimerism after achieving primary engraftment. On the other hand, SGF can be separated in: autologous recovery (AR) where the recovery of host hematopoiesis can only be determined if sequential chimerism studies are undertaken; or in late graft failure (LGF) where definitive pancytopenia occurs with progressive mixed chimerism without subsequent autologous rescue. AR and LGF seems to be different complications with different outcomes. Here, we describe the incidences of AR and LGF of 62 SAA patients transplanted between 1992 and 2011 at HCFMRP. Sequential chimerism was done at D+100, +180, +360 in the first year of transplant, annually after the first year until five years, and in hematological situations where a relapse was suspected. Chimerism was determined by cytogenetics, variable number tandem repeat (VNTR), or blood group serology. Four patients developed AR. Tow of them achieved AR after treatment with CSA + prednisone, other followed treatment with GAL + CSA and the other one did not needed any treatment since he never developed hematologic recurrence (the AR was detected only by the chimerism study in this patient). All four patients are alive and without signs of aplastic anemia 5854, 3209, 4589 and 2133 days after the transplantation respectively. Six patients developed LGF. Tree of them died of sepsis despite the use of CSA + prednisone (two patients) or second transplant with the same donor (one patient). The tree remaining are alive. One of them was treated with a second transplant, other with a bust of donor's peripheral CD34+ cells mobilized with GCS-F and infused without manipulation, and the other with GAL + CSA. These tree treated patients had allo rescue after that treatments. Five of the 62 transplanted patients died of sepsis without engraftment before D+21. None of the 57 patients that survived beyond D+21 coursed with PGF. The incidence of AR and LGF were 7% and 10% respectively. The incidence of AR and LGF, in this Brazilian cohort of SAA transplanted patients was higher them that found by Piccin A, *et al* 2010, in a European cohort of SAA transplanted patients (45 with AR and 20 with LGF in a total of 1270 without PGF - incidence of 3.5% and 1.5% respectively). How this difference is significant or a due to chance is not possible to answer with our data.

**P664**

**Acute and chronic graft-versus-host disease in patients undergoing allogeneic stem cell transplant for severe aplastic anaemia using a conditioning regimen consisting of fludarabine and cyclophosphamide**

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Between January 2004 and June 2011, 105 patients (68 men and 37 women) with a median age of 20 years (range: 2–53) with SAA underwent related HLA identical stem cell transplantation (HSCT). The conditioning regimen consisted of Fludarabine (180 mg/m$^2$ over 6 days), Cyclophosphamide (120 mg/kg over 2 days) + Anti- thymocyte globulin (40 mg/kg over 4 days). Cyclosporine with mini methotrexate was used for GVHD prophylaxis. Graft source included peripheral blood stem cells (96) or G-CSF stimulated bone marrow (9).
One hundred patients engrafted (95.2%) at a median time to neutrophil engraftment of 13 days (range: 8–19) and platelet engraftment of 11 days (range: 7–28). The incidence of grade II-IV acute GVHD was 24% with grade III-IV GVHD in 12% and Grade IV GVHD in 6%. Majority of the patients with grade III-IV GVHD required second line therapy for steroid refractory GVHD. Chronic GVHD occurred in 42% of evaluable patients with equal numbers having limited or extensive chronic GVHD. The 3 year overall survival is 79.1 + 2.3 % with a survival of 90 + 3.6% in patients without GVHD and 63.5 + 10.1% in patients with GVHD.
Though the incidence of acute GVHD is not very high with the use of fludarabine and cyclophosphamide as the conditioning regimen, it still has an adverse impact on survival. Strategies to reduce the incidence of GVHD need to be explored in prospective trials.

**P665**

**TCR alpha/beta and B-cell depleted haploidentical haematopoietic stem cell transplantation in severe aplastic anaemia in children: a case report**

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Background: Allogeneic haematopoietic stem-cell transplantation (AHSCT) from matched sibling donor (MSD) is the therapy of choice for children with severe aplastic anemia (SAA), although graft-versus-host disease (GvHD) or graft rejection remain the major concerns. Children with SAA who lack a MSD and fail to respond to immunosuppressive (IS) treatment should be considered for alternative donor such as from manipulated haploidentical parents. We present a case of child with SAA complicated with lung aspergillosis, treated with haploidentical HSCT (HHCST) with TCR alpha/beta (TCR-a/b) and B cell depletion.
Methods: A 4-year-old female with SAA not responded to IS treatment, presented persistent fever due to lung aspergillosis. In the absence of MSD and prompt available alternative donor, she was subjected to HHCST TCRa/b and B depleted cell from maternal PBSC, using immunomagnetic separation with CliniMACS System (Miltenyi Biotec). Depletion of a/b T cells was 4.5 log. The recovery of CD34+ was 74.6%.The number of infused CD34+,TCRa/b,TCRgamma/delta(TCRg/d) and CD20+ was 11.6X10(6)/kg, 25,092/Kg, 2.57X10(6)/kg, 38,596/kg respectively. Myeloablative conditioning regimen (MCR) included thiotepa (TT) and cyclophosphamide (Cy). IS prophylaxis consisted of rabbit antithymocyte globulin (rATG) and ciclosporin (CsA) (1 mg/Kg i.v. from day -6 to day -1). Despite initial engraftment of absolute neutrophilis count (ANC) and platelets (PLTs) (day +12 and +15 respectively), graft rejection occurred at day +20. After 38 days, a second HHCST TCRa/b and B depleted cell from paternal PBSC was performed. Depletion of a/b T cells was 3.85 log. The recovery of CD34+ was 88.6%. The number of infused CD34+, TCRa/b, TCRg/d and CD20+ was 19.3X10(6)/kg, 94,313/Kg, 5.9X10(6)/kg, 106,000/kg respectively. MCR included TT, Cy and total nodal irradiation. rATG and CsA (3 mg/Kg i.v. from day -6) were used as IS prophylaxis.
Results: Stable ANC and PLTs engraftment occurred from day +14, with clinical and radiological improvement. Mild acute GvHD on the gut occurred at day +30, with complete response to CsA that is gradually tapering. CMV reactivation was controlled with antiviral therapy. Five months later, our patient is still in good clinical conditions with complete donor engraftment and immune reconstitution.
Conclusion: HHCST TCRa/b and B depleted cell is feasible in patients with SAA, due to quick engraftment, reduction of GvHD without impairing pathogen-specific immunity and prompt availability of the donor.

**P666**

**Fludarabine, cyclophosphamide and ATG, with or without low doses of total body irradiation for alternative donor transplants, in children with severe aplastic anaemia. A single-centre experience**

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Objectives: In children with severe aplastic anemia (SAA) undergoing allogeneic hematopoietic stem cell transplantation from unrelated donor (UD-HSCT), treatment-related mortality (TRM) and rejection are major cause of failure. Best results were obtained in patients (pts) receiving HSCT from matched sibling donors (MSD); but because of the frequent unavailability of MSD and the controversial results of immunosuppressive treatment (IST), UD-HSCT is increasingly used. The technique of short tandem repeat (STR), allows better monitoring of the transplant and early intervention in cases at risk of rejection. This study evaluates the outcome of 10 children with SAA undergoing to UD-HSCT after reduced intensity regimen (RIC) based on Fludarabine (FLU), Cyclophosphamide (CY) and ATG.
Methods: Between 1997 and 2010, 10 pts (4 M), median age 10 y, with SAA underwent HSCT from UD after failure of 2 courses of IST. The median interval between diagnosis and HSCT was 24 months. The source of HSCs was bone marrow in 8 and peripheral blood 2. 6/10 was full match donors, while 4 were mis-matched in 1 or more loci. RIC consisted of FLU 120 mg/kg, CY 1200 mg/kg, ATG 15 mg/sqm in 9/10 cases. In 2 case was added 200 cGy Total Body Irradiation. Prophylaxis of aGVHD was made with Cyclosporine (1 mg/kg/die iv from day -5, 2 mg/kg/die iv from day -1, 6-10 mg/kg/die orally for at least 6 months) and short term Methotrexate. Pts received a median of 5.7x10$^8$/kg BM mononucleated cells or 7x10$^6$/kg CD34+ PBSC. The study of complete or mixed chimerism (CC or MC), was performed on BM using STR-PCR technique from the day of take and then every 3 months.
Results: All pts had an initial granulocyte and platelet engraftment, after a median time of 14 and 23 days respectively. There was one case of secondary graft failure with subsequent autologous reconstruction. aGVHD occurred in 2 cases (1 grade II and 1 grade IV) (22%); 4 pts (40%) had a limited chronic GVHD. After a mean follow up of 6.5 years, 9/10 pts are alive and disease free and 8/9 are carriers of CC. The patient who presented secondary graft failure is alive, with a state of MC with autologous prevalence and transfusion independent. One patient died because of grade IV aGVHD.
Conclusion: UD-HSCT in SAA can be currently considered an effective and low toxic procedure even in children. A careful selection of the donor, a low toxic RIC regimen and a high number of infused HSCs can favorably affect the outcome.

**P667**

**Haematopoetic stem cell transplantation for paroxysmal nocturnal haemoglobinuria**

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Aim: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal hematopoetic stem cell disease which can lead to life-threatening complications including intravascular hemolysis, thrombotic events and kidney disease. Improvements in understanding and utilization of high sensitivity diagnostic tests and availability of targeted terminal complement blockade treatment have led to improved awareness and prognosis of PNH.

Patients and Methods: We present data from 6 patients (pts) with PNH who underwent hematopoetic stem cell transplantation (HSCT) in 2001-2011: 4 from match HLA-identical sibling and 2 from matched unrelated donor (MUD). Median time from diagnosis to HSCT was 19 (1-95) months. Indication for allogeneic HSCT was aplastic/hypoplastic bone marrow (2/6 pts), severe course of PNH with hemolytic crisis and/or thrombosis (5/6 pts), transfusion dependency (6/6 pts). Conditioning regimen included total body irradiation (TBI) and cyclophosphamide (CY 60 mg/kg 2 days) and antithymocyte globulin (ATG) in 3 pts, fludarabine (FLU 30 mg/m$^2$ for 4 days) and busulfan (BU 4 mg/kg 2 days) in 1 pt. Before MUD HSCT conditioning regimen contained FLU 30 mg/m$^2$, BU 4 mg/kg 2 days and ATG (2 pts). Graft versus host disease (GvHD) prophylaxis was provided by combination of methotrexate (MTX) + cyclosporin A (CsA) in 5 patients, in 1 patient was MTX replaced with mycophenolate mofetil because of serious gastrointestinal complications. Source of stem cells were dominantly peripheral blood stem cells (5 patients) with median 7.4x10$^8$ nuclear cells/kg (1.6-12.3) and 5.2x10$^6$ CD34+ cells/kg (3.6-5.8).

Results: Engraftment was observed in all patients: median of neutrophil count above 0.5x10$^9$/l, above 1.0 x10$^9$/l and trombocyte count above 50x10$^9$/l were achieved 23 (13-23), 23.5 (14-26) and 23.5 (13-132) days after HSCT. Acute GvHD (intestinal form gr.II-III) developed in 1 patient, 1 has mucosal and ocular signs of chronic GvHD. To date 30.11.2011, 36.5 (0.7-121) months after HSCT all patients are alive and don't require treatment of PNH. In 5/6 evaluable patients complete chimerism i.e. 100% donor hemopoesis and complete disappearance of PNH clone.

Conclusion: Allogeneic HSCT provides effective and safe curable treatment for PNH.

**P668**

**Fludarabine versus no-fludarabine for conditioning in adult patients with idiopathic aplastic anemia for allogeneic haematopoietic stem cell transplantation without total body irradiation from unrelated donor**

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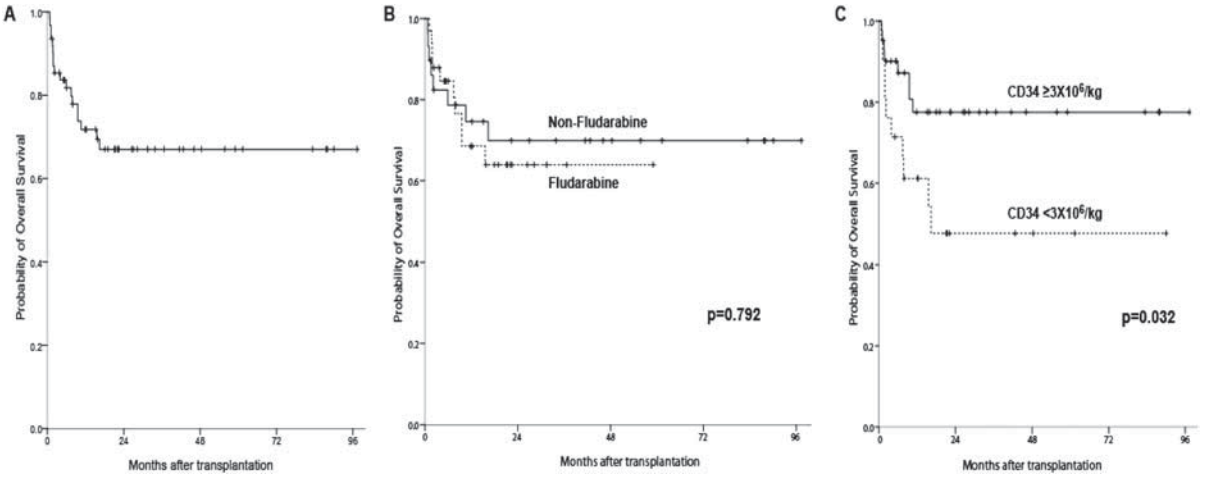
Total body irradiation (TBI) was traditionally used in conditioning regimen for allogenetic hematopoietic stem cell transplantation (alloHSCT) from unrelated donor (UD). More and more patients receive fludarabine-based conditioning regimen without TBI in these days for reducing toxicities of TBI especially in case of UD and elderly patients. We retrospectively investigated the clinical outcomes of alloHSCT from UD without TBI and compared fludarabine-based (fludarabine group) and cyclophosphamide-ATG (non-fludarabine group) conditioning regimen.

Total 62 patients had received non-TBI conditioning regimen for alloHSCT from UD. Male were 53.2% and 80.6% received immune suppression therapy (IST) prior to alloHSCT. Half patients received alloHSCT from HLA full matching donor. Cyclophosphamide (Cy) 200 mg/kg and fludarabine were used for conditioning in 45.2% and 53.2% patients. The median age at alloHSCT was 24.4 (range 14.2-63.6) years. Gender, prior IST, HLA full matching, ABO compatibility and stem cell source were not different between fludarabine group and non-fludarabine group. Cy 200 mg/kg ($p<0.001$) and horse ATG (p=0.002) were mostly used in non-Fludarabine group. Transplantation outcomes including engraftment failure (p=0.960), sinusoidal obstruction syndrome (p=1.000), acute graft versus host disease (GvHD; p=0.874), chronic GvHD (p=0.565), secondary graft failure (p=1.000) and cause of death (p=0.351)were not different between fludarabine group and non-fludarabine group. Time to ANC>500 μ L (p=0.083) and time to platelet>20K/ μ L (p=0.083) were tend to rapid in fludarabine group. Clinical unfavorable factors on overall survival were non-ATG (p=0.058), bone marrow as a stem cell source (p=0.038) and infused CD34+ cells ≤3×10$^6$/kg (p=0.032) in univariate analysis. Only infused CD34+ cells ≤3×10$^6$/kg (HR=2.661; 95% CI 1.049-6.750; p=0.039; Figure 1C) was significant in multivariate analysis. Five year survival rate was 67.0% in all patients and there was no death after 16.349M from transplantation (Figure 1A). Overall survival between fludarabine and non-fludarabine group was not significant (5YSR, 63.9 *vs.* 69.9%; p=0.792: Figure 1B).

In conclusion, alloHSCT without TBI from UD was feasible and there was no difference between fludarabine group and non-fludarabine group.

**[P668]**



**P669**

**Alterations of mesenchymal stromal cellular components in bone marrow niches of patients with aplastic anaemia could contribute to depression of haematopoiesis**

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Background: The hematopoietic bone marrow (BM) microenvironment consists of osteoblastic and vascular niches. During normal hematopoiesis, early hematopoietic stem cells (HSC) are located in the osteoblastic niches, whereas the more mature, proliferating, and migrating HSC are located in the vascular niches. Alterations of theses cellular components are likely to play a role in the pathogenesis of aplastic anemia (AA). The objective of this study was to compare the characteristics of the cellular components of the BM microenvironment in AA patients and normal controls (NC).

Methods: We performed immunohistochemistry for osteopontin, osteonectin, osteocalcin, nestin, stromal cell derived factor-1 (SDF-1, CXCL12), lymphocytes (CD3, CD4, CD8, CD20, CD56), macrophages (CD169) and mast cells, as well as hematopoietic stem/progenitor cells (CD34, CD117) and megakaryocytes, on BM biopsy specimens from 10 AA patients and 10 NC (lymphomas without BM involvement). Cells positive for all the markers except osteocalcin were counted in 10 high power fields (×400, HPF), and their averages per HPF were calculated. Cells positive for osteocalcin were counted on the peritrabecular line on each slide, and corrected by the mean length measured.

Results: The numbers of CD34+ cells, CD117+ cells, and megakaryocytes were significantly lower in the AA than in the NC samples. On the other hand, the numbers of osteopontin+, nestin+, sdf-1+, CD56+ and CD169+ cells were significantly higher in the AA samples. There were significant positive correlations between the number of CD34+ cells and the number of nestin+ and of sdf-1+ cells in the AA samples, but not in the NC samples. However, there were non-significant tendencies for negative correlations between the number of CD117+ cells and the number of nestin+ cells and of sdf-1+ cells in the AA samples.

Conclusions: Higher numbers of mesenchymal stromal cell components in the BM niches in AA may promote the retention and quiescence of HSCs. In addition, they may contribute to reducing the numbers of more mature, proliferating progenitors by inhibiting entry into the cell cycle.

**P670**

**Impact of cytokine gene polymorphisms on risk and treatment outcomes of aplastic anaemia**

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Autoreactive cytotoxic T cells play a key role in the pathogenesis of AA by myelosuppressive cytokines including interferon-gamma (IFN-gamma), tumor necrosis factor alpha (TNF-alpha) and transforming growth factor beta (TGF-beta). The purpose of this study is to determine which single nucleotide polymorphisms (SNPs) in cytokine genes were relevant to AA risk and whether the relevant SNPs were associated with response to immunosuppressive therapy (IST).

Among 84 screened patients, 80 patients confirmed as having acquired AA, and 84 age and sex-matched healthy controls were analyzed consecutively. We genotyped 10 polymorphisms in three cytokine genes (IFNG, TNF, and TGFB1) and FAS gene. We assessed the association between polymorphisms and AA risk, and the association between polymorphisms and response to IST in three genetic models (dominant, recessive, and additive). The IFNG -2353 T allele (dominant model, OR=0.43, p=.012) and TCA haplotype (dominant model, OR=0.50, p=.038) were significantly associated with the development of AA. In addition, this relevant IFNG -2353 T allele and TCA haplotype were related to the response of IST (dominant model, OR=0.076, p=.034). Concerning TGFB1, although its polymorphisms are not related to AA susceptibility, P10L T allele (recessive model, OR=0.18, p=.038) and CT haplotype (dominant model, OR=5.68, p=.038) were associated with response to IST.

In conclusion, this exploratory study concurred with prior studies indicating that polymorphisms in IFNG are related to AA susceptibility. In addition, it was found that polymorphisms in IFNG and TGFB1 are associated with response to IST. Further studies in other population by a larger prospective design are needed to better elucidate the determinants of risk of AA and responsiveness to IST.

**P671**

**Unrelated stem cell transplantation for acquired aplastic anaemia: a donor versus no-donor analysis**

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Background: In the last decade, several studies have reported an improved outcome after hematopoietic stem cell transplantation (HSCT) from a voluntary unrelated donor (VUD) in patients with acquired severe aplastic anemia (SAA). This has led to a marked increase in the utilization of unrelated donor transplantation for patients with marrow failure. However, despite these recent results, the decision to transplant patients who are refractory to immunosuppressive therapy (IST) with VUD remains difficult, the alternative being repeated courses of IST that may be beneficial. In this study, we aimed to shed light on the question of the utility of unrelated HSCT by comparing the outcome of patients with or without a VUD identified.

Methods: We conducted a multicenter donor versus no-donor comparison in 249 pediatric and adult patients with acquired SAA qualifying for a VUD search through the French and Italian donor registries over the 1994-2005 period. In median, the recipient age at diagnosis of SAA was 16 years (range=0-61) and the time interval between diagnosis and donor search initiation was 6 months (range=0-190). HLA matching was defined on low-resolution typing for HLA-A and B class I antigens and high-resolution DNA testing for HLA-DRB1.

Results: Comparing 161 patients in whom a HLA-A, –B and –DR matched donor was identified with 88 patients without such a donor, we show no difference in survival with a median follow-up of 4.3 years from donor search initiation. However, among 179 patients who received IST using anti-thymocyte globulin (ATG), those who were 17 year-old or younger at the time of VUD search initiation had a survival benefit when belonging to the donor group (4-year probability of survival from donor search initiation = 79% ± 6% *vs* 53% ± 10% for the no-donor group, p=0.01). In the same ATG-treated patients (pediatric and adult), the donor group also showed a survival advantage when donor search was initiated within the more recent 2000-2005 study period (4-year survival = 74% ± 6% *vs* 47% ± 10%, p<0.05).

Conclusion: HSCT from an HLA-matched VUD offers a better survival than alternative options in several subgroups of ATG-treated SAA patients. This firstly confirms, in an intent-to-treat manner, the conclusion of a previous prospective study conducted in children with ATG-refractory SAA. It also suggests that, in the more recent period, unrelated donor HSCT would be the best option in ATG-treated patients independently of their age.

**P672**

**Second allogeneic haematopoietic stem cell transplantation for graft failure. Experience of a national bone marrow transplant centre, Tunis.**

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Introduction and objectives: Patients who undergo allogeneic hematopoietic stem cell transplantation (HSCT) may suffer from engraftment failure (EF) or graft rejection (GR). A second HSCT may be the only therapeutic option. We aimed to report associated complications and overall survival.

Patients and Methods: Data on patients who suffered from EF or GR after a 1st HSCT, and who underwent a second HLA –matched related HSCT between April 1999 and December 2010 were recorded.

Results: Twenty one patients underwent a 2nd HSCT. Diagnosis was acquired aplastic anemia in 12 patients, Fanconi anemia (FA) in 3 patients, acute leukemia in 1 patient and supposed FA which was not confirmed thereafter in 5 patients. All received bone marrow (BM) graft for the 1st transplant. The 2nd HSCT was performed for EF in 5 patients and for GR in 16 patients. The median time between the first and the second HSCT was 11 months (1.75- 68). The conditioning regimens used were Cyclophosphamide200-ATGAM90 (n=13) Cyclophosphamide100-ATGAM90 (n=3) and fludarabine containing regimens (n=4). One patient was not conditioned. Seventeen patients received peripheral blood stem cell (PBSC) grafts and four BM grafts from the same donors. The median number of CD 34+ PBSC/kg was 4.5 $x10^6$ (1.65-$10x10^6$). Twenty patients engrafted (95.2%). One patient died on day 0 of the 2nd transplant from DMSO toxicity and was not assessable for engraftment. Graft rejection occurred in 2 patients, respectively on day 30 and day 75 post 2nd transplant and was followed by death from invasive sinusal aspergillosis on day 171 in the 2nd patient. Grade IV acute GVHD occurred in 2 patients and induced death in one of them on day 80. Extensive cGVHD occured in 1 patient after 21 months from transplant. Toxicity related mortality was 14%. After a median follow-up of 50 months (0–138) after 2nd transplant, 18 patients (86%) are alive with a good performans status and a normal blood count.

Conclusion: As it is relatively safe and it offers a real chance to cure, 2nd allogeneic HSCT should be offered to patients who don't engraft or who reject their first graft.

**P673**

**Fludarabine (120 mg/$m^2$) and cycophosphamide (120 mg/Kg), a tolerable and efficient conditioning regimen for matched related allogeneic bone marrow transplantation in severe aplastic anaemia**

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Allogeneic bone marrow transplantation (BMT) is the only curative treatment for severe aplastic anemia (SAA). The main goal of BMT in SAA is to achieve successful engraftment without major complications particularly graft-versus-host disease (GVHD). Radiation therapy (TBI or TN1) promoted efficient engraftment in SAA but was associated with late toxicity and secondary malignancies. Presently, the combination of Antithymocyte Globulin (ATG) and Cyclophosphamide (Cy) given at the total dose of 200 mg/kg is considered the standard conditioning regimen for SAA in the setting of matched related BMT. Because the administration of ATG is associated with significant major side effects and due to its prohibitive cost, ATG had been replaced by Fludarabine (Flu) for immunoablation/ lymphoablation in combination with Cy given at the total dose of 200 mg/kg. We report the experience of our BMT Unit in the use of Flu/Cy: Flu (120 mg/$m^2$) in combination with Cy given at lower total dose (120 mg/kg) in matched related BMT for SAA. Between 1/2009 and 10/2010, 4 patients (pts) who met the criteria of acquired SAA underwent BMT. Median age was 20 years (9-58). There were 3 females and one male. All the pts were treatment naïve. Median time from diagnosis to BMT was 8 months (m) (2-13). Conditioning regimen included Flu: 30 mg/$m^2$/d for 4 days (-7, -6, -5, -4) and Cy: 60 mg/kg/d for 2 days (-3, -2). GVHD prophylaxis consisted of Ciclosporin and Methotrexate (15 mg/$m^2$ on d+1, 10 mg/$m^2$ on d+3 and d+6). Ciclosporin tapering was started on d+120 using 10% dose reduction every 2 weeks. Stem cell source was unmanipulated bone marrow in all pts. The median number of CD34+ cells infused was 2.81x106/kg (1.83-6.8). Engraftment was obtained in all pts. The median time for engraftment of neutrophils and platelets was 16 days (12-25) and 18 days (15-22), respectively. All pts maintained donor chimerism which was evaluated using probes specific for polymorphic DNA sequence (short tandem repeats). Only one patient (pt) developed a grade 3 mucositis. Febrile neutropenia occurred in all pts. Two pts developed a positive CMV antigenemia in blood successfully treated with ganciclovir. Acute grade 3 GVHD occurred in one pt and was steroids sensitive. All the 4 pts are alive with no evidence of disease at 14m+, 18m+, 20m+ and 35m+ respectively. Flu (120 mg/$m^2$)/Cy (120mg/kg) was a well tolerated conditioning regimen with no rejection. However, this needs to be validated in larger series.

**P674**

**Fludarabine-based conditioning for allogeneic marrow transplantation from unrelated donors in severe aplastic anaemia: serious and unexpected adverse events in pre-defined cyclophosphamide dose levels**

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Background and Methods: Since May 2006, the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), sponsored by the U.S. National Institute of Health, has been conducting a prospective Phase I/II clinical trial (BMT CTN 0301) of unrelated donor marrow transplantation in severe aplastic anemia (SAA). SAA patients are eligible if they are < 65 years, have adequate organ function, and have an available unrelated marrow donor matched 7 of 8 HLA-A, B, C, DRB1 loci. All patients receive total body irradiation at 200 cGy, ATG (thymoglobulin: 3 mg/kg IV or ATGAM 30 mg/kg IV daily x 3, days –4 to –2), and fludarabine (30 mg/$m^2$ IV daily x 4, days –5 to –2). The Phase I portion of the trial tested four cyclophosphamide (CY) dose levels: 150 mg/kg (days –4 to –2); 100 mg/kg (days –3 to –2); 50 mg/kg (day –2); and 0 mg/kg. It allowed enrollment of up to six patients at each CY dose level unless toxicity or graft failure boundaries were crossed. In the Phase II portion, patients enroll onto the optimal CY dose level, chosen using adaptive Bayesian criteria ranking CY dose desirability. Early stopping guidelines are used to monitor for graft failure and early transplant-related mortality.

Results: Twenty-one patients accrued to the Phase I portion. CY dose level 0 mg/kg was closed after all three enrolled patients developed secondary graft failure. Phase I data suggested CY dose 150 mg/kg as the optimal dose level for Phase II testing. However, after an additional eight patients were treated at this dose, the level was closed to further accrual due to excess toxicity. Seven of the 14 patients receiving 150 mg/kg of CY (and 7 of the last 8 enrolled) died. Causes of death were (multi)organ failure (n=4), ARDS (n=2), and viral pneumonia (n=1). Bayesian evaluation of the two remaining dose levels indicates similar desirability scores, hence accrual continues at both the CY 100 and 50 mg/kg levels. As of November 15, 2011, sixty-four patients have been enrolled, 17 on the two closed levels, 35 and 12 on the 100 mg/kg and 50 mg/kg levels, respectively.
Conclusions: Early analysis of this trial, prompted by these unexpected adverse events, revealed two important findings: 1) CY dose 0 mg/kg is associated with higher than expected graft failure; and, 2) CY dose 150 mg/kg is associated with excess transplant-related toxicity. To date, CY dose levels 100 and 50 mg/kg have not crossed the graft failure or fatality stopping boundaries and accrual continues.

**P675**

**A comparative study of myeloablative conditioning regimen with cyclophosphamide alone and associated with procarbazine and antithymocyte globulin for allogeneic haematopoietic stem cell transplantation in 196 patients with acquired aplastic anaemia**

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Introduction: Aquired aplastic anemia (AA) is characterized by pancytopenia with a hypocellular, often"empty'' bone marrow. Hematopoietic stem cell transplantation (HSCT) cures AA. We report our experiment of this procedure with retrospective comparative study between two myeloablative conditioning regimen (MCR) protocols.
Materials and Methods: From may 1998 to june 2011, 196 patients (pts) with AA underwent myeloablative allogeneic HSCT from identical sibling donors. Two MCR protocols were used: association of cyclophosphamide 200 mg/m$^2$, procarbazine 37,5 mg/Kg and ATG (Fresenius) 40 mg/kg (C1) for 97 pts (from may 1998 to february 2007), cyclophosphamide alone 200 mg/m$^2$ (C2) for 99 pts (from may 2007 to june 2011). The median age of C1 and C2 pts is 19 years (5,5 to 40) and 21,7 years (4 to 40) (p= 0,9), the sex ratio 2,2 and 1,25 (p= 0,02) and AA was found idiopathic in 81 pts (83,5%) and in 66 pts (66,6%) (p<0,0001) respectively. Interval diagnosis was the same for C1 and C2 group with 11,6 months (1 to 114) and 11,7 (1 to 196) respectively. Multitransfused pts in C1 group (52 pts; 53,6%) was superior than C2 group (18 pts; 18,1%) (p<0,001). The grafts used an peripheral blood stem cells (C1: 88, C2: 99) and bone marrow (C1: 9). All pts had ciclosporine and short course of methotrexate for GVHD prophylaxis. At December 2011 maximal follow-up is 163 months for C1 and 55 months for C2, and minimal 56 months and 6 months respectively.
Results: The time to engrafment is no different for the two groups with a median of 13,9 days (8 to 30) (p=0,5). At December 2011, 68 C1 pts (70,1%) and 84 C2 pts (85%) are alive with a median follow-up of 87 months (56 to 158) and 26 months (6 to 55) respectively. The incidence of acute GVHD is a same for C1 and C2 groups (p=0,68) with 20 pts (24,6%) and 20 pts (21,2%) respectively and the incidence of chronic GVHD was also the same (p=0,68) with 21 pts (29,3%) and 22 pts (25,5%) respectively. The transplant related mortality was observed in 26 C1 pts (26,8%) and 11 C2 pts (11,1%) with significant difference (p<0,001). The incidence of graft failure was identical in the 2 groups, 6 C1 pts (6,2%) and 6 C2 pts (6,1%) (p=0,74). The overall survival (OS) is 64,9% at 13 years, 77,1% at 60 months for C1 group and 82,3% at 60 months for C2 group (p=0,02).
Conclusion: MCR with cyclophosphamide alone at 200 mg/m$^2$ dose seems better than MCR with association in term of TRM and OS.

**P676**

**Allogeneic haematopoietic stem cell transplantation in paediatric patients with Fanconi's anemia and severe aplastic anemia**

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Objectives: To evaluate the outcome of HSCT in pediatric patients with Fanconi anemia (FA) and severe aplastic anemia (SAA).
Methods: We retrospectively analyzed the outcomes of 58 patients with FA (34 male and 24 female) and 41 patients with SAA (27 male and 14 female) who had undergone allo-HSCT between June 1992 and November 2011 in our institution. The patients included in this review were under 15 years. The median age at transplantation was 8 years in FA patients and 11 years in SAA patients. FA patients received fludarabine-based or low dose busulfan-based conditioning regimen and SAA patients received cyclophosphamide plus antithymocyte globulin as conditioning regimen. No radiation therapy was given. Ninety patients received transplants from HLA- matched donors (73 from sibling and 17 from other related donors) and six from one-antigen locus mismatched donor. Moreover, three patients received cord blood stem cell from partially matched unrelated donor. Cyclosporine ± methotrexate was used as Graft-versus-host disease (GVHD) prophylaxis.
Results: The median time to neutrophil engraftment was 11 days (range, 7-41 days) in FA and 13 days (5-22 days) in SAA. The median time to platelet engraftment was 18 days (range, 9-79 days) in FA and 21 days (13-86 days) in SAA. At a median follow-up of 13 months, OS and DFS were 65.2% (SE=6.3%) and 65.6% (SE=7.2%), respectively in FA patients. Seventeen deaths occurred among FA patients. Five SAA patients died at a median follow-up of 47 months. The OS and DFS in SAA patients were 89.8 (SE=4.8%) and 75.9% (SE=7.2%), respectively. The most common causes of death in FA patients were relapse (n=4), graft-versus-host disease (GvHD, n=3) and infection (n=3). Among the patients with SAA, 2 deaths were attributable to infection and one died of GvHD. Thirty nine patients developed acute GVHD in FA and 22 in SAA.
Conclusion: Our good results in SAA patients are comparable in many respects with the results of other studies. Although the outcome obtained from FA patients was not as good as outcome of previous studies, the results have been improved since March 2007. Evaluation of mutations in Fanconi anemia genes among Iranians and their effects on HSCT outcome can help to improve the transplantation outcome.

**P677**

**Haematopoetic stem cell transplantation with fludarabine-based conditioning regimen in Fanconi's anaemia**

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Objective: Hematopoietic stem cell transplantation (HSCT) still represents the only option able to definitively cure the marrow failure associated with Fanconi anemia (FA). Fludarabine-based conditioning regimens, capable of intense T-cell immunosuppression, have been reported to lead to early, stable engraftment with minimal toxicity in patients with several malignant and non-malignant diseases unable to tolerate conventional myeloablative therapy. Here we report the results of the twenty FA patients who underwent HSCT with using fludarabine containing conditioning regimen.

Methods: The study includes patients with FA, confirmed by the presence of multiple chromosome breaks enhanced by incubation with cross-linking agents who underwent an allogeneic HSCT. One patient who had developed acute myeloid leukemia before transplantation excluded from study. Remainig 20 patients were included in the study. The features of donors and the hematopoietic stem cell source of the patients were shown on Table 1. CD 34 selection was performed in 5 (25%) patients. Conditioning regimen include fludarabine (35 mg/kg/day x 5 day), cyclophosphamide (5-10 mg/kg/day x 4 day) and anti-thymocyte globulin. Graft versus host disease (GVHD) prophylaxis was shown on Table 2.

Results: The mean age of patients was 10.5±3.6 y (5.6-17 y) and 14 (70.0%) of them were male. Engraftment was achieved in all 20 pateints The median day of neutrophile engraftment was 13.4±2.7 day (range: 9-19 day). Acute GVHD (≥grade 2) developed in two cases (14.3%), veno-occlussive disease in three cases (15.0%) hemorrhagic cystitis in four cases (%20). Chronic GVHD was not observed in any of the patients. 19 patients (95.0%) survived without disease at a median follow-up period of 24 months (range: 1.5-92 months). One patient who developed poor graft function underwent second HSCT but no improvoment was observed and died due to poor graft function and pulmonary infection after 8.5 month from first transplantation.

**[P677]**

Table 1. Charactersistics of patients

| | |
|---|---|
| Age | 10.5±3.6 y (5.6-17 y) |
| gender (male) | 14 (%70) |
| Donör characterisrics | |
| HLA identical sibling | 13 (65.0%) |
| HLA 1 antigen mismatched sibling | 2 (10.0%) |
| HLA identical other relatives | 5 (25.0%) |
| Source of stem cell | |
| Bone marrow | 13 (65.0%) |
| Peripheral blood | 6 (30.0%) |
| Bone marrow+cord blood | 1 (5.0%) |
| CD 34 selection | 5 (25.0%) |
| Conditioning regimen | Fludarabin (35mg/kg/dayx5day) +Cyclophosphamide (5-10 mg/kg/dayx4 day) +ATG |
| Graft versus host disease prophylaxis | |
| Cyclosporin A+methotrexate | 14 (70.0%) |
| Cyclosporin A* | 5 (25%) |
| Cyclosporin A+methylprednisolone | 1 (5%) |

*CD 34 selection was performed

Table 2. The results of transplantation

| | |
|---|---|
| Neutrophil engraftment | 20 (100%) |
| Neutrophil engraftment day | 13.4±2.7 day (range: 9-19 day) |
| Acute graft versus host disease GVHD (≥grade 2) | 2 (10%) |
| Chronic graft versus host disease | - |
| Veno-occlussive disease | 3 (15.0%) |
| Hemorrhagic cystitis | 4 (20.0%) |
| Survive | 19 (95.0%) |
| Disease free survival | 19 (95.0%) |

Conclusion: Unlike DNA cross-linking agents, fludarabine do not affect the chromosomal integrity of the FA cell. Fludarabine-based protocols have the twin advantage of potent immunosuppression, thus reducing the incidence of graft rejection along with minimal regimenrelated toxicity. The present results based on a small cohort of patients support the use of fludarabine based conditioning regimen for HSCT in patients with Fanconi anemia.

**P678**

**Cyclosporin A response and dependence in children with acquired aplastic anaemia: an update of a multicentre retrospective study with long-term observation**

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Introduction: Immunosuppressive therapy (IST) with antithymocyte globulin and cyclosporin A (CyA) is the standard treatment for children with acquired aplastic anemia (AAA) lacking a matched donor. In our previous study of 42 children with AAA diagnosed from January 1991 to December 1999 and treated with IST we observed an overall survival of 83% at 10 years and a cumulative incidence of relapse of 16% at 10 years that resulted significantly associated with rapid CyA discontinuation. The CyA-dependence without a predictive marker was observed in 18% of responders and the cumulative incidence of MDS/AML was 8% at 10 years, with a significant correlation with both G-CSF cumulative dose and second IST. We concluded that IST with a slow CyA tapering course is an effective treatment with a low-relapse rate in these cases (Saracco *et al*, BJH 2007). In this report we provide an update of our previous study with a 5yrs longer follow up.
Methods and Results: We updated follow up of the 29 alive patients after IST at 1st December 2011. A comprehensive surveillance protocol for relapse, minor PNH clones and MDS/AML evolution was designed and applied.
Overall survival at the last follow-up was 83%. The 29 survivors have been followed for a median of 160 months (range: 49-244): 22 are in remission and off CyA, 3 are still CyA-dependent (median time of CyA treatment 96 months, range 47-98 months), and 1 has mild haemolysis PNH. Two children were lost to follow up. A slow CyA tapering schedule was performed in 84% of patients. Cumulative incidence of MDS/AML was 8% at 12 years; no additional evolution to MDS/AML was observed in our cohort. Analysis of minor PNH clones is under investigation (prospective study ongoing).
Discussion: This updated long-term follow-up of children with AAA confirms that IST with a slow CyA tapering is an effective treatment with a low-relapse rate even if a persistent long-term CyA dependence is still present in 10% of alive patients. Considering the risk of clonal evolution we strongly recommend a focused surveillance strategy.

**P679**

**Follow-up surveillance protocol for relapse, clonal evolution and therapy-related toxicity in children with aplastic anaemia treated with immunosuppression**

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Objectives: Immunosuppressive therapy (IST) with a combination of antithymocyte globulin (ATG) and cyclosporin (CyA) is the first-line treatment for children with aplastic anemia (AA) lacking a family matched donor. However a significant risk of relapse and clonal evolution is present and careful follow up is needed. We describe a comprehensive follow up workup protocol (table 1) to assess clonal surveillance and treatment-related toxicities in children with AA.
Methods and Results: Protocol was applied to a cohort of 28/34 patients (diagnosed between 1990-2010) treated with IST according to EBMT/AIEOP recommendations (horse ATG before 2008). Median age 7.6 years, 18 males, 10 females, 9 very severe AA, 16 severe AA and 3 moderate AA, 14 responders after first IST, 6 responders after 2 IST and 4 non responders (and then transplanted), 2 died of sepsis within 6 months from diagnosis. Among 14 patients responders to first IST marrow biopsy was performed between day +90 and +120 and thereafter marrow aspirate alone was routinely evaluated; in the remaining patients, both marrow aspirate and biopsy were performed every 6/12 months. In only 30% of cases cellularity was concordant on both aspirate and biopsy. Response rate and clonal surveillance were also performed by using flow cytometry evaluation of peripheral CD34+ apoptotic rate (AR) and counts and minor PNH clones analysis (Timeus F, 2005 and 2010). Clonal disease developed in 2 patients with a cumulative incidence of 13% at 20 years. Relapse occurred in 2/14 (14%) concomitantly with a decrease in circulating CD34+ cell absolute count and an increase in AR. Among non responders to IST, analysis for telomerase gene mutations was performed and in 1 child a compound heterozygosity for 2 new TERT mutations was found (Aspesi A, 2010). In the patient 5q- positive at diagnosis, chromosomal abnormality disappeared after remission. Periodical Hb F evaluation during follow up confirmed correlation with stressed haematopoiesis (normalization during stable remission and persistent high levels, up to 24%, among partial or CSA dependent responders). Viral load monitoring showed a viral reactivation (EBV in 3 and CMV in 1) in 4/6 patients treated with first line rabbit ATG. No significant therapy related toxicity was reported.
Conclusion: A comprehensive follow up monitoring protocol is useful to early detect relapse, toxicities and clonal evolution in children with AA and treated with IST.

**P680**

**Second-line ATG-based immunosuppression for refractory aplastic anaemia: predictive factors of response and long-term results**

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Background: Treatment decision making in AA patients failed first line IST with ATG and CsA remains a controversial area. Standard second-line treatment options include repeated IST or HSCT from alternative donor. Thus early identification of patients who are unlikely to respond to IST is an actual problem. The focus of this study is to investigate the results of second line ATG-based IST with particular emphasis on predictors of response.
Methods: In the total cohort of 207 patients we analyzed the outcome in 38 patients (23 M and 15 F, median age 17, 1-60) with refractory moderate (8), severe (17) and very severe (13) AA who received repeated courses of ATG in two centers between March 2000 and June 2011. Refractory AA was defined as FBCs satisfying the criteria for initial diagnosis and/or persistence of transfusion dependence 4 months following first course of ATG. This study includes both retrospective and prospective phases. PNH clones were tested by high sensitive multicolor flow cytometry. End-points of the study were hematological response, overall (OS) and failure-free survival (FFS). The hematological response was evaluated according to the strict response criteria (Camitta B., 2000).
Results: A total of 23 patients (60 %) responded to IST. Nine of 15 non-responders received HSCT. Median follow-up of living patients was 53 months (5-123). The 5-year OS and FFS were 74 % (95 % CI, 59-89 %) and 31 % (95 % CI, 13-48 %). Prognostic factors were analyzed in 31 patients with complete baseline

[P679]

**Table 1. Surveillance Work up for children with AA treated with IST**

**RESPONSE EVALUATION AND CLONAL SURVEILLANCE**

- Full Blood Count (FBC) and reticulocyte, blood smear at each control
- Bone marrow aspirate (cellularity, cytogenetics, colony assay) at day +120 every 6 months during IST, every 12-18 months after off therapy and on demand
- Bone marrow biopsy at day +120 and on demand
- Bone Magnetic Resonance Imaging at day +120 and every 12 months during IST (ongoing study)
- Apoptotic rate circulating CD34+ cells every 3-6 months during IST, every 12 months after off therapy (study 2001)
- PNH clones every 3-6 months during IST, every 12 months after off therapy (study 2008)
- WT1 Immunophenotype on demand
- Hb F% at day +120 and every 6-12 months
- Telomerase gene mutations analysis in non responders (study 2008)

**TREATMENT RELATED TOXICITIES SURVEILLANCE**

- After ATG at each control:
  - Full Blood Count (FBC) and reticulocyte count, creatinine, bilirubin, liver enzymes, glucose, urine exam, C reactive protein;
  - CSA blood levels if the dose is >3mg/kg;
- After ATG every 6 months/12 months
  - Viral studies: Hepatitis A, B and C and HIV (if patient is transfusion-dependent; in case of transfusion-independence, controls must be done 6-12 months after the last transfusion), EBV, Herpes , CMV DNA based monitoring
  - Autoimmunity screening
  - Iron status (iron, trasferrin, ferritin) SQUID/ Fibroscan (depending on iron overload)

data mainly treated in prospective study. Between the responder and non-responder groups, no significant differences in gender, AA severity or interval from diagnosis were observed. Median age of non-responders and responders was 15.7 (1-60) and 18 (2-30) years respectively (p=0.02). Both PNH clone presence and absolute reticulocyte count (ARC) increment after first line IST predicted better response rate and OS. Nine of 10 PNH+ patients with ARC > $25x10^9$/l achieved PR, while none of the 8 PNH- patients with ARC < $25x10^9$/l responded to salvage IST.
Conclusion: These data confirm efficacy of salvage IST in selected AA patients. The absence of PNH clone and the poor ARC increment after first line IST could be considered as candidate markers for early identification of true refractory AA. Further larger studies are warranted for the validation of these prognostic factors in the treatment decision making process, in particular timing of unrelated donor HSCT.

**P681**

**Long-term follow-up of a pilot study with alemtuzumab and low-dose cyclosporine a for aplastic anaemia and single-lineage marrow failures**

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Objectives: We have previously reported (Risitano, BJH 2010;148:791) on our pilot phase II prospective trial (NCT00895739) investigating the anti-CD52 alemtuzumab plus low-dose cyclosporine A (CyA) as immunosuppressive treatment (IST) for aplastic anemia (AA) and single-lineage marrow failures (pure red or white cell aplasias, PRCA and PWCA). Here we report the update and the long-term follow up of our single-center experience.
Methods: Twenty-eight patients entered the study (18 untreated): 13 (8) SAA, 13 (9) PRCA and 2 (1) PWCA. Median age was 51 years (range 25-87). The IST regimen consisted of subcutaneous alemtuzumab 3-10-30-30-(30) mg, in consecutive days, (total dose 103 mg for SAA, 73 mg for PRCA and PWCA), followed by oral CyA 1 mg/kg. Anti-infectious prophylaxis included valganciclovir (for 3 months) and bactrim.

Results: The treatment was completed in all patients without any serious adverse event; the median follow up is now 42 months. Severe lymphocytopenia developed immediately and lasted several months (especially for CD4+ T-cells). Nevertheless, infectious events were infrequent and clinically mild, with exception of 1 fatal sepsis and 1 progressive multifocal leukoencephalopathy (occurring in a PRCA patient relapsed with metastatic thymic carcinoma, during salvage chemotherapy). No CMV disease nor EBV-related disease was observed; 5 patients developed asymptomatic CMV reactivation cleared by pre-emptive valganciclovir. The response rates were 77% (38.5% CR) for AA and 84.5% (61.5% CR) for PRCA patients; both PWCA achieved durable CR. Current stable remission were achieved in 38.5% of AA and 23% of PRCA; the majority of long-term responders have received an additional dose of alemtuzumab to sustain the response. Long-term failures were due to refractory relapses (15% for AA and 7.5% for PRCA) or to clonal evolution (15% for AA and 23% for PRCA). Overall survival at 42 months was 73% for AA (all deaths due to refractory disease), and only 40% in PRCA (most deaths due to clonal evolution or associated comorbidities).
Conclusion: Long-term follow up of patients treated with alemtuzumab confirms that this agent has a remarkable efficacy for the treatment of immune-mediated marrow failures, with acceptable safety profile and promising response rates. Even if the current regimen has been not optimized yet, alemtuzumab-based IST may represent the best alternative to h-ATG, and could be considered for large head-to-head prospective comparative studies.

## Autoimmune Diseases

**P682**

**Long-term outcomes of autologous haematopoietic stem cell transplantation in severe autoimmune diseases: an extended analysis of the EBMT database 1996-2011**

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Objective: To analyze the durability of the responses after Autologous Haematopoietic Stem Cell Transplantation (AHSCT) for severe autoimmune diseases (SAD) and whether the Overall Survival (OS) and Progression Free Survival (PFS) remained stable over time, we aimed to evaluate the longer outcome of patients (pts) originally described (1) and to identify potential prognostic factors.
Methods: AD pts treated by first AHSCT in European phase I-II studies from 1996 up to 2011 and reported to the EBMT registry were included. Transplant regimens followed the international consensus statements. Primary endpoints were Overall Survival (OS), Progression Free Survival (PFS) and the 100 days Transplant Related Mortality (TRM).
Results (median): Among 1177 AD patients (63 % female, median 35 years(yrs)) treated by first AHSCT, we identified mainly: 465 multiple sclerosis (MS), 216 systemic sclerosis (SSc), 96 systemic lupus erythematosus (SLE), 84 rheumatoid arthritis (RA), 72 juvenile arthritis (JIA), 70 Crohn's Disease (CD) and 39 hematological immune cytopenia. The respective global 5 and 7yrs OS were 86% and 83%, varying widely according to AD type with respectively 92 % and 90 % for MS, 75% and 70 % for SSc, 76 % and 71 % for SLE. The respective global 5 and 7 yrs PFS were 43 % and 36 % and varied from 46% and 37 % for MS, 50 % and 39 % for SSc, 34 % and 30 % for SLE. When analysing the subgroup of pts transplanted from 2005 to 2010, following the 2004 Consensus statement concerning cardiotoxicity occurring during HSCT in the treatment of SAD, with special reference for MS and SSc, the OS, PFS and TRM remains stable, directly related to each disease type. The overall day 100 TRM was 5% and has not varied in the last 5yrs (2006-2011: 5%, 2001-2005: 4% and 1996-2000: 7%) depending on AD category.
Conclusions: This extended report from the ADWP EBMT data base confirms that AHSCT can induce sustained remissions in patients with SAD refractory to conventional therapy with durable responses at 5 up to 7 yrs after AHSCT. Early identification of patients with adequate fitness for AHSCT in accordance with current EBMT guidelines (2) is warranted.
(1) AHSCT for autoimmune diseases: an observational study on 12 years experience from the EBMT ADWP, D Farge *et al* Haematologica 95, no. 2 (2010): 284-292
(2) HSCT in severe autoimmune diseases: updated guidelines of the EBMT.Snowden J *et al* Bone Marrow Transplant. 2011 Oct 17, doi: 10.1038/bmt.2011.185

**P683**

**Fifteen years of stem cell transplantation for autoimmune diseases at an Ottawa hospital**

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Stem cell transplantation (HSCT) has been used to treat patients with severe refractory autoimmune disease (AID) based on pioneering pre-clinical animal studies and resolution of coincident AID following HSCT for malignancy. Fifty-four patients with AID have undergone HSCT at the Ottawa Hospital. Three patients underwent HSCT from 1996 to 2000, 17 patients during 2001 to 2005, 26 patients during 2006 to 2010 and 8 patients during 2011. Three additional patients had successful autologous stem cell collections with cyclophosphamide (CTX) and growth factor mobilization but did not proceed to HSCT due to stabilization of AID (n=1), progressive AID (n=1) and severe complications of chemotherapy (n=1). AID indications included: multiple sclerosis (n=32), myasthenia gravis (n=7), Crohn's disease (n=4), rheumatoid arthritis (n=3), chronic inflammatory demyelinating polyneuropathy (n=2), scleroderma (n=2), stiff person syndrome (n=2), neuromyelitis optica (n=1), and systemic lupus erythematosis with immune thrombocytopenic purpura (n=1). Four of the patients had AID coincident with a hematological malignancy. Three of these patients underwent allogeneic HSCT from matched related (n=2) or matched unrelated (n=1) donor, while one patient received an unmanipulated autologous graft. The remaining 50 patients underwent autologous HSCT for refractory AID using CTX and growth factor mobilized peripheral blood stem cell grafts. Allogeneic recipients received unselected bone marrow grafts while 49 of the autologous grafts were immune depleted using CD34(+) CliniMACS immunomagnetic selection. Condition regimens included Busulphan with CTX (n=44), CTX with total body irradiation (TBI) (n=5), CTX (n=4) and Etoposide with Melphalan and TBI (n=1). Antithymocyte globulin was administered to 48 patients. Graft versus Host disease occurred in 2 of the 3 allograft recipients. Overall survival is 92% with the longest follow-up being 169 months. Two of the deaths (3.7%) were due to regimen related toxicity occurring -3 and 62 days from HSCT. Two deaths occurred 24 and 30 months after HSCT due to progression of concurrent lymphoma (n=1) and progression of scleroderma (n=1). There have been no deaths among the last 29 HSCT recipients. HSCT remains a viable therapy with an acceptable regimen-related mortality for patients with severe refractory AID.

**P684**

**Haematopoietic SCT in severe autoimmune diseases: analysis of UK outcomes from the British Society of Blood and Marrow Transplantation (BSBMT) data registry 1997-2009**

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The BSBMT Data Registry was analysed for SCT in severe autoimmune diseases (SADs) in the UK 1997-2009 inclusive. 70 SCTs were performed in 69 patients, with 55 autoSCT (median 38 yrs, 64% female) and 15 alloSCT (median 10 yrs, 53% female), comprising 0.22% of overall UK SCT activity and 5.8% of EBMT activity for SADs. Indications were predominantly rheumatological (74%), while MS was rare (4%). Median time from diagnosis was 5 yrs. PBSC predominated as SC source in autoSCT (90%), with both BM (40%) and PBSC (60%) used in alloSCT (6 related, 9 unrelated). Conditioning was mainly cyclophosphamide-based (85%) ± ATG (53%) in autoSCT and fludarabine-based (87%) in alloSCT. Engraftment was generally prompt; neutrophils >0.5 x$10^9$/L median 12 days for autoSCT, 13 days for alloSCT; platelets>20 x$10^9$/L 11 days for autoSCT, 16 days for alloSCT. Prospective clinical trial enrolment was reported in 20%.

Overall survival (OS) at 1 and 5 yrs was 85% (73-92%) and 78% (63-87%) in autoSCT, and 87% (61-99%) and 65% (36-84%) in alloSCT respectively. Progression free survival (PFS) at 1 and 5 yrs was 51% (37-64%) and 33% (20-47%) in autoSCT and 80% (50-93%) and 65% (36-84%) in alloSCT respectively. 100 day and 1 yr non-relapse mortality (NRM) was 13% (6-23%) and 15% (7-25%) in autoSCT and 7% (1-41%) and 13% (2-35%) in alloSCT respectively, mainly from infection and pulmonary toxicity. Secondary autoimmune diseases were reported in 6% of autoSCT.

For first autoSCT, univariate analysis confirmed significant associations between OS and age (5 yr OS 95% in the 18-39 yr age group *vs* 67% in <18 years and 64% in >40 yrs, p=0.03), in males *vs* females (OS at 5 yrs 95% versus 70%, p=0.02) and with connective tissue diseases (CTD) having inferior OS and NRM compared with non-CTD indications (5 yr OS 56% *vs* 88%, p=0.0006; NRM 29% *vs* 8%, p=0.04). Otherwise there were no significant differences in OS, PFS or NRM for the time period of SCT (1996-2003 *vs* 2004-2009) or time from diagnosis of SAD to SCT (<5 years versus >5 yrs).

This is the first reported national analysis of SCT in SADs in the context of the translational and developmental phases of this approach to poor prognosis and refractory SADs. Use of SCT as salvage treatment resulted in sustained responses, albeit with significant toxicity. Early identification of poor risk but reversible SADs with adequate fitness for SCT in accordance with current EBMT guidelines, along with greater involvement in prospective studies, is warranted.

**P686**

**Low baseline complement levels, autoantibody persistence and delayed thymic reactivation are risk factors for development of relapses after haematopoietic stem cell transplantation for refractory SLE**

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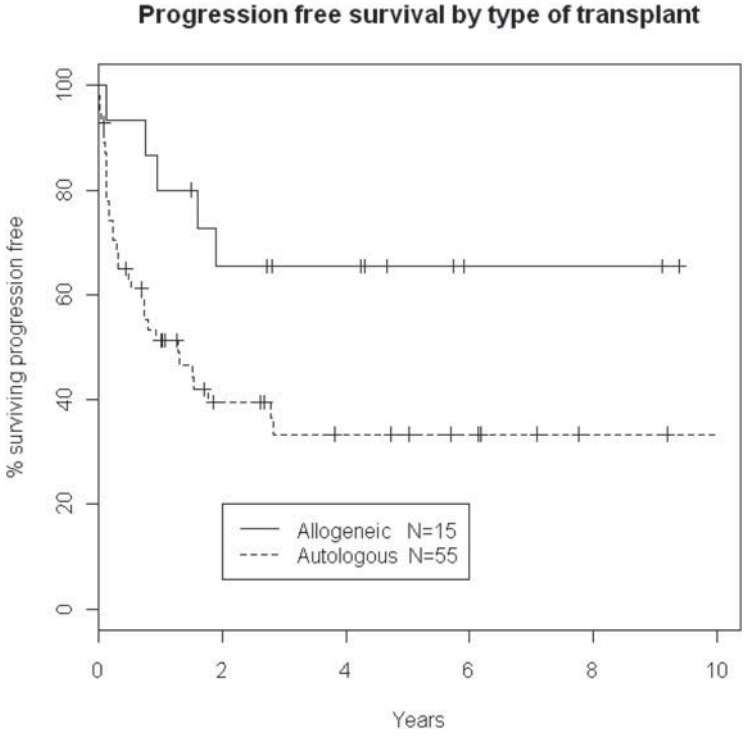
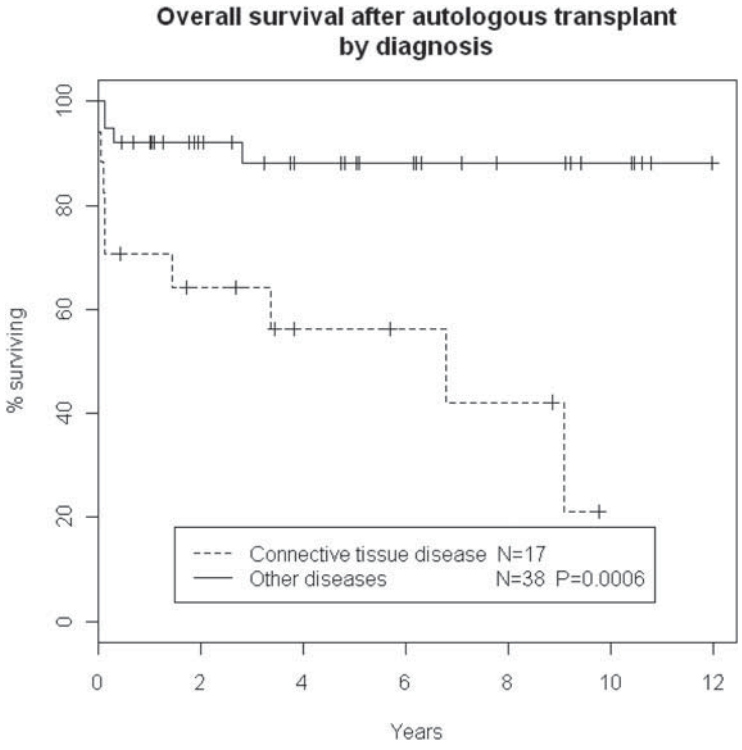
Objectives: Clinical trials have indicated that immunoablation followed by autologous hematopoietic stem cell transplantation (HSCT) has the potential to induce long-term, treatment-free remissions in systemic lupus erythematosus (SLE). However, disease flares may occur in a subset of these patients post-transplantation. We longitudinally analyzed the immune reconstitution of these patients to identify predictive cellular or serologic markers for long-term remissions.

Methods: Since 1998, eight patients with SLE underwent CD34-HSCT after conditioning with CYC (200mg/kg) and rabbit-ATG (90mg/kg) as part of a monocentric phase I/II clinical trial. Autoantibody titers were evaluated with ANA-immunofluorescence and ELISA, and peripheral T- and B lymphocyte subsets immunophenotyped using multicolor flow cytometry.

**[P684]**



[P684]




Findings: Clinical remission (SLEDAI ≤ 3) could be achieved in all patients accompanied with disappearance of anti-dsDNA antibodies and protective antibodies in serum. Two patients died due to transplant-related infections. From the remaining six patients, three patients are in long-term clinical remission for up to thirteen years after HSCT, while three patients suffered a relapse of SLE at 18, 36 and 80 months post-transplantation, respectively. Patients with early relapses (≤36 months) had lower baseline complement levels (C3 ≤ 67 mg/dl), showed persistence of antinuclear antibodies and had slower repopulation of CD31+ CD45RA+ thymic-derived CD4+ T cells after HSCT when compared to long-term responders. In contrast, antinuclear antibodies in the patient with late relapse (80 months) initially disappeared but later redeveloped, preceding the lupus-flare by 4 years.
Conclusion: We identified low baseline complement levels, persistence of antinuclear antibodies and delayed thymic reactivity as risk factors for development of lupus flares after HSCT. Since ATG-mediated apoptosis is complement-dependent we conclude that low serum complement is directly associated with incomplete depletion of autoreactive memory cells in these patients. Our data suggest that normalization of serum complement levels before conditioning should be considered in SLE patients, either through substitution or transplantation during a period of lower disease activity.

**P687**
**Effects of OAZ in regulating B-cell proliferation by mesenchymal stem cells from patients with systemic lupus erythematosus**
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Objective: Allogeneic mesenchymal stem cell transplantation (MSCT) has shown some benefits in patients with refractory systemic lupus erythematosus (SLE). However, the underlying mechanism is still unclear. Our data indicate that Olf1/EBF associated zinc finger protein (OAZ), a novel lupus susceptibility gene down-regulated after MSCT, is involved in the production of antinuclear antibody (ANA). Because this gene is highly expressed in MSCs, the role of OAZ in MSC-B cell regulation is explored.
Method: Study protocol was approved by the hospital's Ethics Committee. MSCs isolated and expanded after culturing for 3 passages from bone marrow of 4 female SLE patients, were incubated with siRNAs targeting OAZ or non-targeting sequence. Three days later, cells were collected for measuring mRNA levels of OAZ and ID1-3, downstream genes of OAZ, using quantitative real-time polymerase chain reaction (qPCR) and levels of cytokines and chemokines in cultured supernatants were detected by ELISA. Splenic B cells from C57BL/6 mice were purified using anti-CD43 antibody, co-cultured with SLE MSCs at 10:1 ratio in the presence or absence of OAZ siRNAs, and then harvested for the detection of proliferation by using BrdU assay.
Result: Silencing OAZ in SLE MSCs 1) significantly reduced mRNA levels of OAZ and its downstream ID1-3 by 67.9 ± 7.2% and ~50% respectively, 2) significantly increased expression levels of CCL2, a MSC-derived chemokine involved in plasmablast proliferation, in both mRNA and protein of cultured supernatants, and had no effects in IL-21 levels. LPS stimulated proliferation of mouse B cells was not affected by co-culturing with human MSCs at 1:10 ratio (28 ±0.4% *vs* 31 ± 0.8%, n= 3 and $p > 0.05$), but was impaired (18 ± 1.6%, $p<0.05$) by the addition of siRNAs targeting human OAZ gene in the co-cultures. Interestingly, when anti-CCL2 neutralizing antibody (1 ng/ml) was added to MSC-B cell co-cultures, the ability of B cell proliferation was fully restored.
Conclusion: Down-regulating of OAZ in MSCs increases CCL2 levels and inhibits B cells proliferation in MSC-B cell co-cultures, implicating a role of OAZ in SLE MSCT.

**P688**

**Mesenchymal stem cell transplantation ameliorates refractory cytopenia in patients with systemic lupus erythematosus**

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Objective: Our previous data showed that mesenchymal stem cells (MSCs) had potentially therapeutic effects on autoimmune diseases including systemic lupus erythematosus (SLE), based on their immunosuppressive properties and low immunogenicity. This study focused on the roles of allogeneic MSCs transplantation (MSCT) in SLE patients with refractory cytopenia.
Methods: Thirty-five SLE patients with refractory cytopenia were enrolled in a MSCT trial. Hematological changes of pre- and post-transplantation were evaluated. Mechanisms for MSCT effects focused on the analysis of percentage of peripheral blood regulatory T cells (Treg) and Th17.
Results: Our results showed that in 35 SLE patients, 24 patients had leukopenia, 24 with anemia and 24 with thrombocytopenia. The median follow-up periods after MSCT was 21 months (range 6-45 months). Significant improvements in blood cell count were found after MSCT for most of patients, in parallel with the decline of disease activity. Clinical remission was accompanied by increased Treg and decreased Th17 cells. Two patients died of uncontrolled disease recurrence after infection and adverse events related to transplantation were not observed.
Conclusion: The data suggested that MSCT resulted in hematological improvements in SLE patients with refractory cytopenia, which might be associated with reconstitution of Treg and Th17 cells. Further studies for long-term larger-scale and the mechanism explorations for MSCT effects are necessary.

**P689**

**Enhanced apoptosis and senescence of bone marrow-derived mesenchymal stem cells from patients with systemic lupus erythematosus**

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Objective: Previous studies have indicated that bone marrow mesenchymal stem cells (BMSCs) from patients with systemic lupus erythematosus (SLE) exhibit impaired capacities of proliferation, differentiation, secretion of cytokines and immune modulation. In this study, we aimed to investigate whether apoptosis and senescence of BMSCs from SLE patients were dysregulated.
Methods: BMSCs were isolated from bone marrow of SLE patients and healthy controls by density centrifugation and adhesive culture *in vitro* TNF-alpha was added to detect its effect on the activation of apoptosis. The apoptosis of BMSCs was evaluated by TUNEL assay and Annexin V-FITC/PI Apoptosis Detection. Real-time PCR technique was used to determine the gene expressions of Fas, Bcl-2, Bax, Bcl-w, Caspase 8 and TNFR or without with TNF-alpha. Cytochrome C was detected by immunocytochemistry. Western blot was used to detect the expressions of Fas, TNFR and Caspase 8. The expressions of Fas, Bcl-2 and the activity of Caspase 8 were detected by flow cytometry. Meanwhile, serum levels of FasL and TNF-alpha were measured by ELISA.
Results: The frequencies of apoptotic and ageing BMSCs from SLE patients were significantly increased in culture when compared with those of healthy controls. Notably, levels of Bcl-2 expression in BMSCs from SLE patients were markedly deceased both at mRNA and protein levels. When BMSCs were induced to apoptosis *in vitro* stimulated by TNF-alpha the Bax and Caspase 8 expressions in BMSCs from SLE patients were significantly inceased at mRNA levels. The activity of Caspase 8 was enhanced in BMSCs from SLE patients. More cytochrome C positive pellets in the cytosolic fraction were detected in BMSCs from SLE patients compared with healthy controls. The expressions of Fas and TNFR1 on BMSCs from SLE patients were significantly upregulated compared to healthy controls as well as serum level of FasL and TNF-alpha. Moreover, intracellular ROS levels of BMSCs from SLE patients were higher than those of healthy controls characterized with the activation of PI3K/AKT/FoxO3 signaling pathway.
Conclusion: Our results have demonstrated increased apoptosis and senescence in BMSCs from SLE patients, which may be associated with the pathogenesis of SLE.

**P690**

**Allogeneic mesenchymal stem cells transplantation in severe and refractory systemic lupus erythematosus**

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Objective: To assess the long-term efficacy and safety of allogenic bone marrow or umbilical cord derived mesenchymal stem cells transplantation (MSCT) for patients with severe and treatment-refractory systemic lupus erythematosus (SLE). ClinicalTrials.gov Identifier: NCT00698191.
Methods: A single-arm trial involved 87 refractory SLE patients, aged from 12 to 56 years old. Disease duration was 37.5 months (range, 2 to 264 months). 51 of 87 patients were each given a total of 0.6~2.4 gm of CYC intravenously. The other 36 patients received no CYC due to bad physical conditions at baseline. Thirty-one and 51 patients were infused with bone marrow (BM) or umbilical cord (UC) derived MSC, respectively. One million cells per kilogram of bodyweight were administered intravenously. The clinical manifestations and laboratory parameters were compared pre- and post-MSCT, with a mean follow-up of 27 months (range, 12 to 48 months). Adverse event was monitored all the time during and post-MSCT.
Results: During the 4 years follow up, the overall rate of survival was 94% (82/87). Five patients died 5, 6, 6, 10 and 18 months post-MSCT respectively, due to uncontrolled infection or disease relapses. Disease-free survival was 28% at 1 year (23/83), 31% at 2 years (12/39), 42% at 3 years (5/12) and 50% at 4 years (3/6). Rates of relapse were 12% (10/83) at 1 year, 18% (7/39) at 2 years, 17% (2/12) at 3 years and 17% (1/6) at 4 years. The overall rate of relapse was 23% (20/87). Significant improvements in SLEDAI score, serum albumin and complement 3 were examined at 1, 2, 3, and 4 years post transplantation. Renal function assessed by 24-hour proteinuria improved 1, 2, 3 and 4 years after MSCT. Serum creatinine and urea nitrogen declined significantly 1 and 2 years after MSCT. Glomerular filtration rate (GFR) was detected and significant improvement was found. No differences were found in disease remission, SLEDAI score, proteinuria and serum albumin levels between those with or without CYC pretreatment, those infused with BM or UC MSC. During 4 years follow up, no transplantation-related adverse event was observed for all the patients. For 93% (76/82) patients, dose of glucocorticoid was tapered to less than 10mg per day, and dose of immunosuppressant was tapered to maintenance level for 60% (49/82) patients at the last follow up.
Conclusion: Allogeneic MSC transplantation is a safe and effective therapeutic option for severe and refractory SLE patients.

**P691**

**Pulse high-dose cyclophosphamide in malignant multiple sclerosis may result in successful functional recovery and stem cell mobilisation to enable safe delivery of AHSCT**

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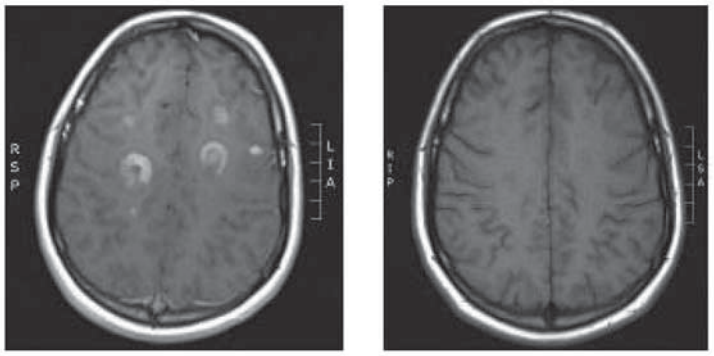
Current EBMT guidelines1 recommend autologous stem cell transplant (ASCT) for refractory malignant multiple sclerosis

[P691]



MRI T1 post-contrast images:
Right - May 2011
Left - November 2011 after high dose cyclosphosphamide and AHSCT

(MS), but advise against AHSCT in severe disability and respiratory muscle compromise. We describe a patient with rapid onset malignant MS, refractory to conventional treatments and requiring high dependency support, in whom repeated pulse high dose cyclophosphamide (CY) resulted in sufficient functional recovery and stem cell mobilization for safe delivery of AHSCT.
A 21-year-old woman presented with progressive sensory and motor disturbance to both arms and legs in April 2011. Neuro-imagining demonstrated evidence of demyelination with enhancement and CSF had 47 white cells and was positive for oligoclonal bands. Two courses of IV methylprednisolone were given, followed by 7 sessions of plasma exchange. Despite this, symptoms progressed with increasing weakness, ataxia, nystagmus and oscillopsia; repeat imaging demonstrated multiple new white matter lesions in the brain and longitudinally extensive myelitis. Clinical deterioration continued and within 2 months she was tetraplegic and requiring ventilatory support, at which time she was treated with alemtuzumab and IV methylprednisolone without immediate response.
A decision was made to intensify treatment with CY 2g/m$^2$ and Granulocyte-colony stimulating factor in July 2011, following which stem cell harvesting was performed. Clinical improvement was noted, although the patient remained bedbound, dependent on ventilatory support and MRI continued to show new lesions and pathological enhancement in the brain and spinal cord. Repeat CSF examination revealed 10 white cells. Plasma and CSF neuromyelitis optica antibodies were negative. A further pulse of CY 2 g/m$^2$ was given, after which MRI appearances improved, with loss of enhancing lesions. Subsequently, in November 2011, underwent CY 200 mg/kg + anti-thymocyte globulin, and AHSCT. Routine toxicity, including neutropenic sepsis, was successfully managed and engraftment was prompt. Continued neurological improvement has been observed; she has good functional control of her upper limbs and is able to stand with assistance. Repeat MRI scanning shows a stable lesion load with no post-contrast enhancement.
In conclusion, pulse CY may improve functional status in severely disabled patients with malignant MS permitting successful delivery of AHSCT.
1. Snowden JA, *et al*. Bone Marrow Transpl 2011, E-pub, Open access.

**P692**

**Allogeneic haematopoietic stem cell transplantation in refractory neuromyelitis optica spectrum disorders**

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High-dose immunosuppressive chemotherapy with autologous Hematopoietic Stem Cell Transplantation (ASCT) can induce remissions in patients with severe treatment-refractory Autoimmune Diseases. Unfortunately, despite transient control of disease activity, relapses can occur. Allogeneic SCT (alloSCT) has the potential of cure through the Graft-versus-Autoimmunity effect.
Here we report a stable clinical remission after alloSCT in two patients affected by severe forms of Neuromyelitis Optica (NMO), a neurological autoimmune disorder associated with a poor prognosis and often resistant to current standard treatments.
Both treated patients had aggressive NMO, positive for aquaporin-4 antibodies (AQP4). Upn#1 is a 30 yrs old male affected by severe relapsing longitudinally extensive trasverse myelitis (LETM). Upn#2 is a 28 yrs female with optic neuritis (ON) and LETM. Both had received multiple lines of treatment without benefit, including corticosteroids, cyclophosphamide, rituximab, natalizumab, alemtuzumab, plasma exchange, thiotepa/cyclophosphamide and BEAM/ATG/CsA with ASCT rescue.
Upn#1 was transplanted from a HLA-id sibling, upn#2 from a 9/10 MUD. Conditioning regimen consisted of full-dose treosulfan, fludarabine, rituximab and ATG-Fresenius. GvHD prophylaxis was MTX and CsA (upn#1) or mycophenolate and rapamycin (upn#2). Hematopoietic recovery occurred within day 30, achieving full donor chimerism in both patients. There was one episode of febrile neutropenia and one of CMV reactivation, both responsive to medical therapy; no serious adverse events were reported. None of the patients experienced neither acute nor chronic GvHD. Neurological function was markedly improved in both treated patients (EDSS score dropped from 6 to 5 in upn#1 and from 8.5 to 7.5 in upn#2), and both are relapse-free at last follow up (31 and 18 months respectively). MRI did not evidence any new lesions. Importantly, AQP4 antibodies became negative in both patients. Immune reconstitution was characterized by preponderance of naïve B cells (CD38+/CD27- range 86-91% *vs* 43-47% in controls) and

increased output of recent thymic emigrants (CD4+/CD45RA+/CD62L+/CD31+ 72-87% *vs* 61-96%) and T regulatory cells (CD4+/CD25+/FoxP3+/IL-7Ralpha- 3.6-4.9% *vs* 1.7-5.2%). These findings suggest that alloSCT may be beneficial in patients with aggressive forms of NMO, by renewing the immune repertoire of T- and B-cells, thus reducing disease activity and arresting disability progression.

**P693**

**Autologous haematopoietic stem cell transplantation in aggressive forms of neuromyelitis optica (NMO) and NMO spectrum disorders**

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Background: Neuromyelitis Optica (NMO), an inflammatory and demyelinating autoimmune disorder of the central nervous system, previously considered to be a severe variant of Multiple Sclerosis (MS), is characterized by recurrent attacks of optic neuritis (ON) and by longitudinally extensive trasverse myelitis (LETM). More recently NMO has been recognized as a distinct disorder with positivity for anti-aquaporin 4 (AQP4) antibodies, a poorer prognosis and an unsatisfactory response to most treatments currently in use for MS.

Methods: We evaluated the outcome after high dose immunosuppressive chemotherapy with autologous stem cell transplantation (ASCT) of four patients with aggressive forms of NMO, unresponsive to conventional therapies. All patients had a LETM and 1 also a ON. Patients had a median of 36 years, 3 females and 1 male. Previous treatments included high dose steroids, Cyclophosphamide (CTX), Rituximab, Natalizumab, Plasma Exchange (PEX), Azathioprine, Methotrexate. Mobilization was obtained with CTX 4 gr/mq at day 0 followed by granulocyte colony stimulating factor 5 mcg/Kg/day from day +2 to stem cell harvest. HSC harvest was not manipulated before cryopreservation. Three patients received a Thiotepa-CTX based conditioning regimen before ASCT, while one patient a BEAM/ATG/Cyclosporin.

Results: Mobilization was successfull in all cases, with a median of $23{,}23 \times 10^6$ collected and $6{,}1 \times 10^6$ infused CD34+cells/Kg. Hematopoietic recovery was documented in all patients, with a median of 13,5 days for neutrophil engraftment and 11 days for platelet. All patients developed febrile neutropenia, two a CMV reactivation, without serious adverse events. Two patients experienced a neurological worsening during the aplasia. All patients are alive. Relapses were observed in all patients within 1 year after ASCT, requiring other treatments, included high dose steroids, Rituximab, PEX, Alemtuzumab and for two patients allogeneic stem cell transplantation (Allo-SCT).

Conclusions: ASCT has been unsatisfactory to maintain long term disease remissions and to reduce disability progression in four patients with aggressive NMO spectrum disorders. Autoimmune Diseases Working Party have launched a retrospective survey of ASCT for NMO and NMO spectrum disorders.

**P694**

**Mesenchymal stem cells and autoimmune diseases: the case of multiple sclerosis**

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Objectives: In multiple sclerosis (MS), an autoimmune disease of the central nervous system (CNS) characterised by progressive demyelination of brain and spinal cord axons, mesenchymal stem cells (MSCs) have been proposed as cell therapy for their ability in modulate disease processes. MSCs may modulate immune response, as it has been shown in animal models, and may act through the secretion of many substances that provide support for the damaged CNS. The use of autologous MSCs will bypass the risk of the use of an allogenic MSC Bank, therefore we study the characteristic of MSCs isolated from MS patients compared to healthy donors (HD) as a crucial step before transplantation.

Methods: In the present work we characterised bone marrow (BM) MSCs isolated from 12 MS patients and 20 HD. We investigated phenotype by Flow cytometry, proliferation and colony forming unit (CFU) number, differentiation ability by osteogenic and adipogenic *in vitro* differentiation, immunomodulation capacity by inhibition of T cell proliferative response, toll like receptor (TLR) expression by real time (RT) PCR, cytokine production by ELISA and cell signalling pathways by Milliplex Millipore (ERK #44-611, p38 #46-610A, creb#46-631A, jnk#46-613A, STAT1#46-655A).

Results: MSCs isolated from MS patients show a significant ($p<0.003$) increased production of IP10, a T cell recall chemokine, at several *in vitro* passages together with increased phosphorylation of p38, CREB, JNK, and STAT1. All tests were performed before and after TLR4 stimulation. Interestingly, the basal level of activation for all these factors was higher in MS MSCs, except for STAT1. At the same time, MSC immunomodulatory action was not altered.

Conclusions: The increased production of IP10 in MS MSCs goes together with an altered basal activation state and an increased phosphorylation after TLR4 stimulation of factors forming signalling cascade responsible of IP10 production and secretion. This may represent a disease marker or an effect of previous therapies or both. Our data indicate that an attentive evaluation of cells is mandatory in MS and autoimmune diseases as stem cell therapies may form an important part of the therapeutic approach in the future.

**P695**

**Estimation of the effectiveness of autologous haematopoietic stem cell transplantation in very severe cases of paediatric secondary-progressive multiple sclerosis**

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Effectiveness of Autologus hematopoietic stem cell transplantation (AHSCT) in case of very severe forms multiple sclerosis (MS) is undoubted. The optimal conditioning regimen is the combination of cyclophosphamide and ATG/Campath. There are only a small number of AHSCT cases in very severe refractory secondary-progressive pediatric MS according the international experience. T-regulatory cells (T-regs) play a significant role in immunological tolerance maintenance and inhibition of autoimmune aggression.

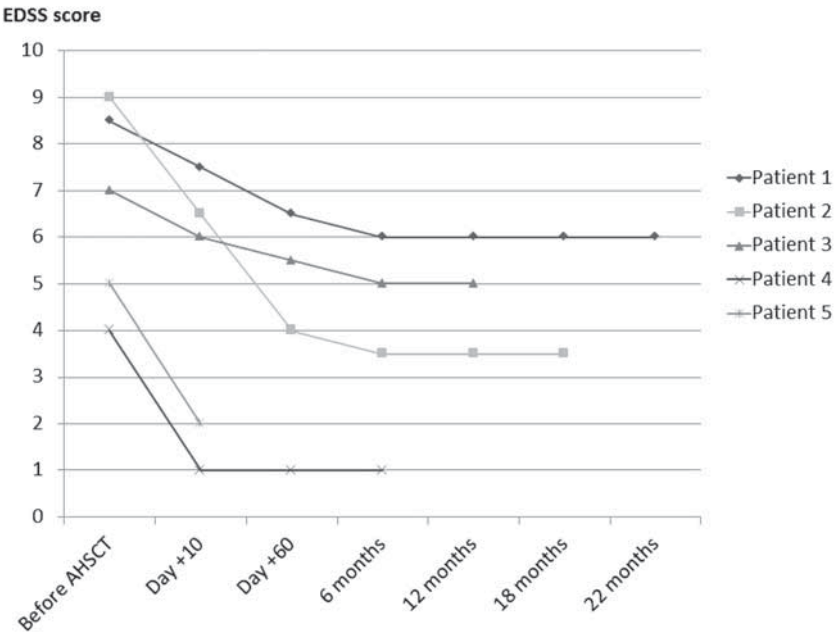
Objective: Evaluation of the AHSCT effectiveness in very severe refractory cases of secondary-progressive pediatric multiple sclerosis. T-regs level assessment at a patients with MS in the context of AHSCT.

Patients and Methods: The survey includes 5 cases of pediatric MS duration 3-4 years treated with corticosteroids, interferon-beta and plasmapheresis with negative dynamics on this therapy.

Patient's EDSS score and dynamics described on picture 1.

All the patients had approved MS diagnosis with significant neurological disturbances, severe inflammatory lesions with the contrast accumulation on MRI and positive oligoclonal bands in cerebrospinal fluid. Mean EDSS – 7.0 points.

[P695]




Procedures: Stem cell mobilization: Cyclophosphomide, G-CSF. Conditioning: Cyclophosphomide 200 mg/kg and ATG "Fresenius" 40 mg/kg or equivalent. Myeloinfusion on day 0. G-SCF stimulation from day +5.
Intravenous immunoglobulins 10% (IVIGs) administration according the IgG serum level.
Study design: 1. Clinical examination; 2. MRI; 3. Lymphocytes subpopulation assessment; 4. T-regs level evaluation; 5. Toxicity control.
Results: All the patients showed fast improvement during early post-AHSCT period. Maximal EDSS improvement – 5.5 points, mean – 3.5 points. In patient 4 – to 1.0 points. All the patients in remission now. Maximal observation period – 22 months. Expressed positive dynamics registered on MRI too. T-regs assessment showed its level increasing and stabilization after the AHSCT. Usage of IVIGs allows protecting patients against the infections during the cytopenic period and improving neurological status in prolonged period.
Conclusion: AHSCT is the effective way of autoimmune inflammation reduction and successful approach for treatment of very severe secondary-progressive pediatric MS. This approach is not only life-saving method, but significantly improves quality of patient's life. In-time AHSCT can minimize the disability level.

**P696**

**Early versus late autologous haematopoietic stem cell transplantation in multiple sclerosis patients: analysis of treatment outcomes**

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High-dose immunosuppressive therapy with AHSCT offers promising results in the treatment of MS patients. Reduced intensity conditioning regimens (mini-AHSCT) is a way to improve the balance between benefits and side effects of this treatment approach. The patient selection criteria for this treatment modality are still unclear. We report the results of a prospective phase II open-label single center study with the analysis of the safety and efficacy of mini-AHSCT in MS patients depending on the type of transplantation.

Ninety-five patients with MS: secondary progressive – 35 patients, primary progressive –15, progressive-relapsing – 3, and relapsing-remitting – 42, were included in this study (mean age - 34.5, male/female – 36/59). The conditioning regimen included reduced or modified BEAM. Median EDSS at base-line was 3.5 (range 1.5–8.5). Forty-two patients underwent early (EDSS 1,5–3,0), 50 patients - late (EDSS 3,5–6,5), and 3 patients - salvage (EDSS 7,0–8,0) mini- AHSCT. Neurological assessment using EDSS was performed at baseline, at discharge, at 3, 6, 9, and 12 months after transplantation, every 6 months thereafter up to 48 months, and then at yearly intervals. Progression-free survival (PFS) was calculated using the Kaplan-Meier method.
No transplant related deaths were reported. The mobilization and transplantation procedures were well tolerated. 39 patients after early transplantation and 51 patients after late/salvage transplantation with the follow-up period of ≥ 9 months were included in the clinical outcome analysis. After early transplantation all the patients responded to the treatment; after late/salvage transplantation all the patients, except one, were stable or had disease improvement.
At long-term follow-up (median 46 months) in the group after early transplantation the clinical response was classified as an improvement in 46% of patients, 48% of patients remained stable, and 6% of patients progressed. In the group after late/salvage transplantation (median 43.5 months) 32% of patients achieved improvement, 37% – stabilization, and 31% progressed. The estimated PFS at 5 years was 92% in the group after early mini-AHSCT versus 73% after late/salvage mini-AHSCT.
Thus, mini-AHSCT is an effective treatment option in MS patients. The results of our study support the feasibility of early AHSCT in MS patients. Multicentre cooperative studies should be done to optimize the treatment protocol of mini-AHSCT in this patient population.

**P697**

**Haematopoietic stem cell transplantation for systemic sclerosis: Brazilian experience**

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Systemic sclerosis is a life-threatening autoimmune disease with progressive skin fibrosis and internal organ involvement.

Treatment has limited efficacy and severe cases are associated with mortality rates of up to 50% in 5 years. Hematopoietic stem cell transplantation (HSCT) has been studied as a therapeutic alternative for severe and refractory cases. Twenty-five patients with diffuse cutaneous disease, mean age of 32.9 (16-58) years, 21 females, mean non-Raynaud disease duration of 2.8 (0.5-10) years, were mobilized with 2g/m$^2$ cyclophosphamide plus G-CSF. Peripheral blood hematopoietic stem cells (HSC) were harvested through apheresis and cryopreserved. In sequence, the patients received high dose cyclophosphamide (200 mg/kg) plus rabbit anti-thymocyte globulin (4.5 mg/kg), followed by infusion of autologous, non-selected HSC. One patient died after mobilization due to aspiration pneumonia associated with very severe scleroderma. A second patient died 22 days after HSCT due to bacterial sepsis. One patient abandoned follow-up after successful mobilization, with improvement of skin fibrosis. Twenty-two patients were followed for a median of 24 (6-60) months. Modified Rodnan Skin Score decreased significantly ($p<0.0001$), beginning 6 months after transplantation. Hemoglobin-adjusted Carbon Monoxide Diffusing Capacity (DLCO) and forced vital capacity (FVC) rates persisted stable throughout follow-up ($p>0.05$). One patient relapsed 6 months after HSCT, and one patient was non-responsive to HSCT. Two patients had a systemic lupus erythematosus (SLE) overlap, with SLE relapse after HSCT (cytopenias). Both were successfully treated with Rituximab. In conclusion, HSCT is a feasible treatment for severe cases of systemic sclerosis, with partial and sustained reversal of skin involvement in most cases and stabilization of pulmonary function.

**P698**

**Autologous non-myeloablative haematopoietic stem cell transplantation without CD34+ selection for refractory Crohn's disease: the Milan experience after 5 years**

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Background and aims: Over the past 15 years, the use of autologous haematopoietic stem cell transplantation (HSCT) in severe autoimmune diseases resistant to conventional treatment has constantly increased. Here we report an update on our experience with unselected stem cell autotransplantation in refractory Crohn's disease (CD).
Patients and Methods: Since November 2005, ten patients (4 males, 6 females) with active moderate-severe CD (median CDAI 340, range 236-395; four with perianal disease), all refractory or intolerant to multiple drugs including anti-TNF-alpha agents and various immunosuppressive agents, were enrolled. Median age was 35 years (22 to 46). Unselected PBSCs were collected after mobilisation with CTX 1.5 g/m$^2$ and G-CSF 10 µg/kg. Conditioning regimen included CTX 50 mg/kg on days –5 to –2 and rabbit ATG 2.5 mg/kg on days –4 to –2. Toxicity, clinical remission (CDAI < 150), endoscopic remission (SES-CD) and extramucosal response (ultrasound sonography [US]) were assessed after mobilisation and at 3, 12 months and then every year after stem cells reinfusion.
Results: No improvement was confirmed after mobilisation (median CDAI 364, range 201-404). Neutrophils and platelets engraftment occurred at a median of +12 and +9 days, respectively. At the third month following transplantation, all patients were in clinical remission with a CDAI less than 150 (median 91, range 56-132) despite discontinuation of all medications. After a median follow-up of 56 months (range 23-68), clinical remission was maintained in 80%, 50%, 40%, 30% and 30% of the evaluable patients at the 1st, 2nd, 3rd, 4th and 5th year after HSCT, respectively. Complete mucosal healing was observed in 50% and 60% after 3 and 12 months, respectively, but maintained in only 30% at their last visit. Perianal fistulas closure was observed in 3/4 patients. No deaths or life-threatening infections occurred. Adverse events included a perianal abscess after mobilisation in one patient, pleural and pericardial effusions in another, BK virus-related macrohaematuria in one case, acute pielonephritis in another patient, all rapidly resolved with conservative treatment.
Conclusions: Although relapses have occurred, we observed tsreatment-free remissions for as long as 5 years. We conclude that unselected CD34+ cells transplantation can induce and maintain both clinical and endoscopic remission in refractory CD patients.

**P699**

**Autoimmune thyroiditis in diabetic patients treated with autologous haematopoietic stem cell transplantation**

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Secondary autoimmune diseases are common complication after HSCT for autoimmune diseases and can be present in about 10% of transplanted patients. Diabetes type 1 is an autoimmune disorder in which autologous hematopoietic stem cell transplantation is applied. Diabetes is often accompanied by various autoimmune disorders such as thyroid disorders, celiac disease or vitiligo. As high as 40% of diabetic patients have second autoimmune disorder.
We report here the occurrence of autoimmune thyroid disorders in diabetic patients that underwent HSCT at our institution (n=15). The patients were treated with conditioning regiment containing cyclophosphamide and rabbit antithymocyte globulin. The median follow up is 26 months. During that period we identified 7 cases of autoimmune thyroid disorder (46% of the patients) with presence of anti-thyroid peroxidase and anti-thyroglobulin antibodies. Only one patient had secured diagnosis of autoimmune thyroiditis prior to transplantation. All patients had thyroid-stimulating hormone within normal values prior to HSCT, but the anti-thyroid peroxidase and anti-thyroglobulin antibodies were not measured for most of the patients before transplantation. Time range from transplantation to thyroid dysfunction was 5,5 to 15 months. Five of seven patients were females. The autoimmune thyroid disorder so far required treatment in 2 patients (1 Graves-Basedov disease, 1autoimmune thyroiditis). Of note is that 4 of 7 patients with autoimmune thyroid disorder returned to insulin dependence but at lower doses than before transplantation.
Conclusion: The patients after HSCT for diabetes might be prone to autoimmune thyroid disorders – however at the rates typical for diabetes. The routine testing for anti-thyroid antibodies should be included in screening and follow up tests in this group of patients.

## Stem Cell Mobilization and Graft Engineering

**P700**

**Comparable efficacy and safety of biosimilar and originator G-CSF in patients undergoing haematopoietic stem cell mobilisation**

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Objectives: Recombinant granulocyte colony-stimulating factor (G-CSF) is widely used to mobilise haematopoietic stem

cells. Biosimilar filgrastim is now available in Europe. No differences were observed between biosimilar filgrastim (n=40) and a retrospective cohort of patients receiving originator filgrastim for stem cell mobilisation in a previous comparison, although no safety findings were reported (Lefrère *et al*. Adv Ther 2011;28:304-10). We compared the efficacy and safety of a biosimilar filgrastim (Zarzio®, Sandoz Biopharmaceuticals) with originator filgrastim (Neupogen®, Amgen) in patients with haematological malignancies.
Methods: A total of 108 patients were included in this study, 59 of whom were female (49 male), with an overall median age of 51 years (range 19-69). Patients had multiple myeloma (n=46), Hodgkin's lymphoma (n=26), non-Hodgkin's lymphoma (n=28) or other diagnosis (n=8). Median time from diagnosis to mobilisation was 10 months (range 3-122). After administration of mobilising regimens (primarily high-dose etoposide, high-dose cyclophosphamide, intermediate-dose Ara C or ESHAP), patients were randomised to a standard daily 10 µg/kg dose of Zarzio® (n=54) or Neupogen® (n=54).
Results: Median duration of G-CSF administration was 8 days with both Zarzio® (range 4-17) and Neupogen® (range 4-14). Both groups had a median of one apheresis with a median time until first apheresis of 11 days. There were no statistically significant differences between groups in the median (range) number of mobilised CD34+ cells/µL in peripheral blood (Zarzio®, 62.0 [2-394]; Neupogen®, 47.5 [2-370]) or the number of CD34+ cells/ kg body weight (Zarzio®, 9.1 [0-23]; Neupogen®, 9.4 [6-48]). Five patients (9%) in each group did not mobilise sufficient CD34+ cells. The adverse event profile was comparable between the Zarzio® and Neupogen® groups, with similar occurrence of neutropenic fever (9 *vs* 11 patients) and bone pain (8 *vs* 6 patients).
Conclusion: Zarzio® demonstrated similar efficacy and safety as the reference filgrastim (Neupogen®) in haematopoietic stem cell mobilisation in patients with haematological malignancies.

**P701**
**Quantitation of CD34+ stem and progenitor cells: comparison of a new direct volumetric flow cytometric method with the standard, beads-based flow cytometric method**
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Objectives: Following apheresis and cyropreservation, the precise enumeration of CD34+ hematopoietic progenitor cells (HPC) as key parameter of graft quality is mandatory for the clinical course after transplantation. Currently, flow cytometry based on beads technology performed as single-platform (SP) technique represents the "gold standard" for such determinations. We compared a classic SP method (M1) for enumeration of CD45+ or CD34+ cells with a new device allowing direct volumetric measurements (M2) of HPC.
Methods: Fresh samples, 14 from mobilized peripheral blood (PB) and 9 from apheresis products, and 13 samples from frozen-thawed HPC grafts were analyzed for CD34+ cells, CD45+ cells, and in frozen-thawed samples for viability by M1 and in parallel by M2.
Results: Correlation analysis for CD34+ cells revealed a significant ($p<0.01$) correlation between both techniques with $r = 0.95$, $r = 0.933$, and $r = 0.929$, respectively, for each material. Additionally, measured median numbers of CD34+ cells/µL did not differ comparing results resulting from M1 with results from M2 for the different analyzed materials (fresh PB: 41.5 versus 41; apheresis products: 2,680 versus 2,355; frozen-thawed samples: 2,170 versus 2,399). For analysis of CD45+ cells/µL, the correlation was significant ($p<0.01$) for fresh PB samples and for frozen-thawed samples between M1 and M2 measurements. Furthermore, the median number of CD45+ cells/µL evaluated with the different techniques did not differ between the three analyzed materials. In frozen-thawed samples, the analysis of viability was comparable and without differences for M1 and M2.
Conclusions: The results of this study demonstrate that a direct volumetric analysis of CD34+ cells/µL or CD45+ cells/µL is feasible in different materials and that the results do not differ from the results derived from the standard SP method. This volumetric technique may represent a new and cheaper approach to further standardize HPC analyses.

**P702**
**Successful mobilisation, harvest, and transplantation of autologous haematopoietic progenitor cells more than 10 years after total body irradiation and chemotherapy-based conditioning for first transplantation**
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Objectives: Relapse of Non Hodgkin`s lymphoma (NHL) occurring several years after autologous hematopoietic progenitor cell transplantation (aHPCT) rises the question of the availability of HPC for a second transplantation (Tx). Data regarding feasibility of mobilization and harvest of HPC after first myeloablative conditioning and HPCT are rare. Therefore, we report two cases of successful mobilization, harvest, and for one of the two patients Tx of HPC more than 10 years after first Tx.
Case Reports: In two patients with a NHL diagnosed in 1996 (a 47-years-old man) and 1997 (a 23-years-old woman) initial chemotherapy was followed by harvest of autologous HPC after mobilization with Dexa-BEAM (dexamethasone, BCNU, etoposide, cytosin-arabinoside, melphalan) and G-CSF. Both patients were successfully transplantated with aHPC after total body irradiation (TBI) and cyclophosphamide (Cy) in 1996 and 1998, respectively. Relapse occurred in both patients in 2011, 13 and 15 years after first Tx. After reinduction of another complete remission, mobilization therapy was given. This consisted of Cy (3 g/sqm) for the female patient and due to previous toxicity of R-DHA (rituximab, dexamethasone and high-dose cytosine-arabinoside) for the male patient followed by G-CSF. A total of 5.5x10E+06 CD34+ cells/kg of bw (female patient) and 8.1x10E+06 CD34+ cells/kg of bw (male patient) were harvested in one apheresis processing 5.5 times (female) and 3.6 times (male) the total peripheral blood volume of the respective patient. Analysis of clonogenic growth in samples of satellite tubes of the grafts after freezing and thawing revealed results comparable to data from patients without previous aHPCT. Until now, only the female patient was transplanted a second time. She received the complete dose of harvested aHPC after conditioning with BEAM. The patient exhibited regular engraftment parameters with respect to a neutrophil count > 500/µl on day +11 and a platelet count > 20,000/µl without transfusion support on day +11 after Tx. The patient could be discharged from hospital on day +13 after Tx in good clinical condition and is in complete remission 3 months after Tx.
Conclusions: In patients with relapse of NHL more than 10 years after myeloablative conditioning and autologous Tx a new attempt to mobilize HPC after reinduced remission is feasible. Even a TBI based previous therapy does not exclude successful remobilization, harvest, and retransplantation in such patients.

**P703**

**Retrospective analysis of bone marrow grafts: comparison of unmanipulated bone marrow grafts with manipulated bone marrow grafts due to AB0 blood group incompatibility between donor and recipient**

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Objectives: Transplantation (Tx) of allogeneic hematopoietic progenitor cells (HPC) is a therapeutic option for patients with hematological malignancies. For bone marrow (BM) grafts, the sufficient dose of HPC for successful engraftment is usually defined by the number of nucleated cells (NC) per kg of body weight (bw). In case of AB0 blood group incompatibility between donor and recipient, red cell depletion or plasma reduction are necessary with the result, that NC are decreased and are no longer a reliable parameter of graft quality.

Methods: We performed a retrospective analysis of graft parameters of consecutive HPC transplantation with BM at two German centers (Kiel, Münster).

Results: During the analyzed time period, at total of 131 BM Tx were performed. The key parameters age of recipient, volume of the graft, number of NC/CD45+ cells per kg of bw, and number of CD34+ cells/kg of bw were analyzed.

Results: At the time of analysis, one or more key parameters were available for a total of 101 Tx, 67 performed with a manipulated (GI) and 34 with an unmanipulated graft (GII). The median age of recipient was comparable between GI and GII. The median volume of the graft of GI before manipulation and of the graft of GII were comparable (1,020 ml and 1,094 ml, respectively). The final median volume (140 ml) of the graft in GI after manipulation was significantly ($p<0.01$) smaller compared with the other volumes. Neither the median number of NC/CD45+ cells per kg of bw nor the median number of CD34+ cells/kg of bw were different between GI before manipulation and GII (3.1x10E+08 versus 3.1x10E+08, 2.8x10E+06 versus 3.4x10E+06). In addition, the median number of CD34+ cells/kg of bw in GI after manipulation (3.0x10E+06) was not different compared with the above values. But the number of NC/CD45+ cells per kg of bw decreased significantly ($p<0.001$) to a median of 1.05x10E+08 when compared with the values of GI before manipulation and the values of GII. In addition, this was below the accepted threshold dosage of 2-3x10E+08 NC per kg of bw for Tx.

Conclusions: Red cell depletion and/or plasma reduction in AB0 blood group incompatible BM Tx led to a significant decrease of graft volume and of the number of NC/CD45+ cells per kg of bw. The number of CD34+ cells/kg of bw was not influenced by such manipulations and seemed to be the more reliable parameter for such BM grafts. Although, a potential correlation to engraftment should still be analyzed.

**P704**

**Analysis of intraapheresis recruitment of haematopoietic progenitor cells after mobilisation with plerixafor**

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Objectives: Successful mobilization of sufficient or even optimal amounts of hematopoietic progenitor cells (HPC) is necessary for transplantation after high-dose therapy. In patients who do not mobilize sufficient numbers of HPC, the addition of Plerixafor offers a new therapeutic option.

Methods: We analyzed a series of patient undergoing mobilization with plerixafor for intraapheresis recruitment (IR) of HPC. IR was defined as difference of the total number of CD34+ cells/kg of body weight (bw) in the harvest and the total number of CD34+ cells/kg of bw in the peripheral blood (PB) before apheresis. Data from 98 aphereses of 55 different patients being mobilized with chemotherapy and/or G-CSF and Plerixafor were retrospectively analyzed regarding number of CD34+ cells in PB before apheresis and number of CD34+ cells in the graft.

Results: 27 of the patients were female and 28 were male. The median age of the patients was 56 years (range: 14-75 years) Patients` diagnosis were multiple myeloma (n=23), Non Hodgkin's lymphoma (n=27), Hodgkin's disease (n=2), germ cell tumor (n=1), Ewing's sarcoma (n=1), and B-CLL (n=1). A median of 2 aphereses (range: 1-5) per patient were performed processing a median of 4 times (range: 1.4–7.7 times) the total PB volume. The median number of CD34+ cells/µl in PB before apheresis was 15 (range: 2-148). A median amount of 1.77x10E+06 CD34+ cells/kg of bw (range: 0.14x10E+06 - 17.5x10E+06) was harvested per apheresis. The median IR per apheresis was 0.87x10E+06 CD34+ cells/kg of bw (range: -0.66x10E+06 to 7.40x10E+06). A nonparametric correlation (according to Spearman) analysis between the number of processed total PB volume and the IR revealed a weak ($r=0.36$) but significant ($p<0.01$) correlation.

Conclusions: The presented data demonstrate, that the addition of plerixafor in poorly mobilizing patients can induce an IR of HPC during harvest. In addition, expanding the apheresis size seems to further improve and optimize the potential yield of HPC by increasing the intraapheresis recruitment.

**P705**

**Use of plerixafor in first stem cell mobilisation: a decisional algorithm based on the number of circulating CD34+ cells before apheresis**

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Autologous hematopoietic stem cell (HSC) transplantation is a treatment strategy for restoring hematopoietic function in patients with non-Hodgkin's lymphoma (NHL), multiple myeloma (MM) and/ or Hodgkin's lymphoma.

We conducted a monocentric retrospective study on the mobilization procedure to 1) determine the place of plerixafor in the mobilization strategy and to 2) define an algorithm of mobilization procedure. Twenty- four patients were analyzed, 9 NHL and 15 MM, with a median age of 56,4 years [42-66].

For the first 20 patients, the mobilization strategy was the use of G-CSF and chemotherapy (group 1) (n=17) or G-CSF alone (group 2) (n=3). All these patients failed to mobilize enough PBSC. The median of circulating CD 34+ cells before apheresis was 4.25µ/L [0-19] and 4µ/L [0-11] respectively for the group 1 and 2. The characteristics of these 20 patients have shown predictive factors associated to poor mobilization. For these 20 patients, we decided to use the combination of G-CSF followed by daily plerixafor for the second mobilization procedure.This second attempt to mobilize PBSC was successful for 17 patients (85%). The median number of circulating CD34+ was 35.5 [2-300] on day 2 of plerixafor. The number of CD34+ cells collected for transplantation was 6.05 [0 to 43.08 x $10^6$ CD34+/kg]. The median number of apheresis sessions for these 20 patients was 1.6 [1-3] (Figure 1a-b).

According to the results of the 20 first patients and based on the results of Micallef *et al* (ASH 2009, abstract 3211), we designed a algorithm for the decision to use G-CSF + plerixafor in first mobilization procedure (Figure 2).

The following 4 patients with predictive factors to poor mobilization (75% were >60 y, 75% received previous intensive chemotherapy, 50% have a platelet count <100x$10^9$/L before

apheresis, and 100% have principally a number of circulating CD34+ cells insufficient to proceed to apheresis after G-CSF alone).
With this strategy, at day 5, patients received plerixafor and the mobilization was successful for 100% of the patients [4.57-8.14 x $10^6$ CD34+/Kg] with a low number of apheresis sessions.
In conclusion, this decisional algorithm seems to be useful to optimize the of PBSC by using G-CSF and plerixaflor. Especially, patients with predictive factors to poor mobilization and principally a peripheral blood CD34+ cell count < 10/µL after 5 days of G-CSF could be good candidate to plerixafor in first stem cells mobilization.

**P706**

**Chemomobilisation and plerixafor in 'poor mobilizers' – a single-centre experience**

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Introduction: Mobilization failure is a major issue in heavily pretreated patients involving 15.2% of our cases with conse-

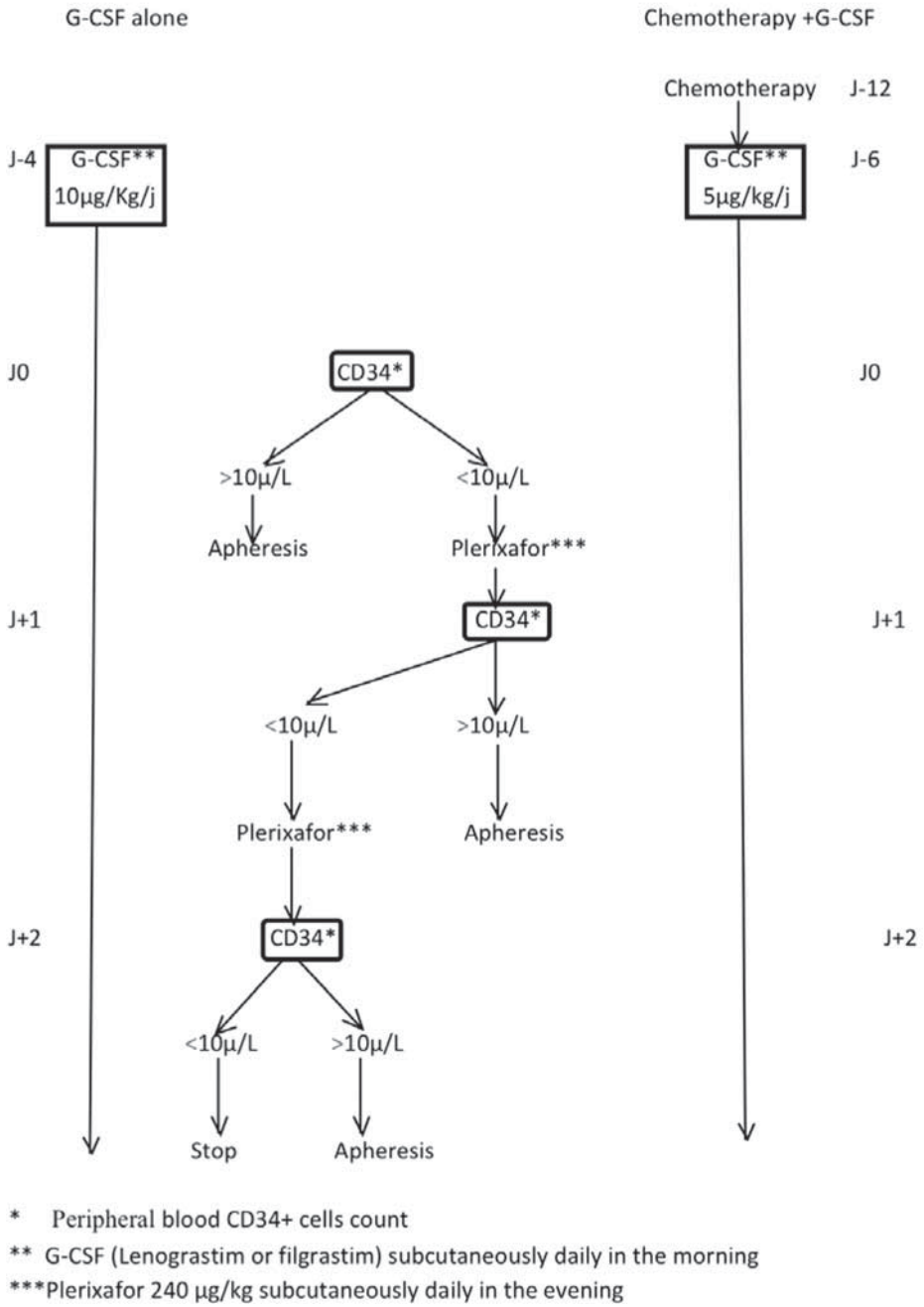
**[P705]** **Figure 1a.** Characteristics of the mobilization procedure and results of 20 first patients

| Age (years) | Type of disease | Mobilization procedure | Circulating CD34+ cells µ/L before apheresis | Number of apheresis sessions | Collected CD34+ cells $10^6$/kg |
|---|---|---|---|---|---|
| | | G-CSF | 5 | 2 | 0.63 |
| 49 | NHL | **G-CSF+plerixafor** | 3 | 1 | 0 |
| | | G-CSF+chemotherapy | 3 | 1 | 0 |
| 48 | NHL | **G-CSF+plerixafor** | 1 | 2 | 0,8 |
| | | G-CSF | 4 | 1 | 0.33 |
| 45 | NHL | **G-CSF+plerixafor** | 4 | 3 | 0.73 |
| | | G-CSF | 4 | 4 | 1.75 |
| 48 | NHL | **G-CSF+plerixafor** | 11 | 2 | **2.87** |
| | | G-CSF+chemotherapy | 4 | 3 | 0,31 |
| 48 | NHL | G-CSF+plerixafor | 26 | 1 | **2,98** |
| | | G-CSF+chemotherapy | 2 | 1 | 0 |
| 50 | NHL | **G-CSF+plerixafor** | 19 | 1 | **4.23** |
| | | G-CSF+chimiotherapy | 3 | 1 | 0 |
| 49 | NHL | **G-CSF+plerixafor** | 7 | 3 | **3,65** |
| | | G-CSF+chemotherapy | 3 | 1 | 0 |
| 61 | NHL | **G-CSF+plerixafor** | 16 | 2 | **4,2** |
| | | G-CSF+chemotherapy | 10 | 2 | 1.22 |
| 63 | NHL | **G-CSF+plerixafor** | 10 | 1 | **4.89** |
| | | G-CSF+chemotherapy | 0 | | |
| 66 | NHL | **G-CSF+plerixafor** | 273 | 1 | **14,83** |
| | | G-CSF+chemotherapy | 0 | | |
| 58 | NHL | **G-CSF+plerixafor** | 28 | 2 | **2,46** |
| | | G-CSF+chemotherapy | 0 | | |
| 48 | NHL | **G-CSF+plerixafor** | 26 | 1 | **3,12** |
| | | G-CSF+chemotherapy | 10 | 2 | 0.62 |
| 62 | NHL | **G-CSF+plerixafor** | 7 | 2 | 0.70 |
| | | G-CSF+chemotherapy | 0 | | |
| 45 | NHL | **G-CSF+plerixafor** | 12 | 2 | 2,88 |
| | | G-CSF+chemotherapy | 3 | 1 | 0 |
| 49 | MM | **G-CSF+plerixafor** | 11 | 1 | 1.9 |
| | | G-CSF+chemotherapy | 15 | 3 | 0.99 |
| 65 | MM | **G-CSF+plerixafor** | 45 | 2 | **9.54** |
| | | G-CSF+chemotherapy | 0 | | |
| 58 | MM | **G-CSF+plerixafor** | 42 | 1 | **8.62** |
| | | G-CSF+chemotherapy | 0 | | |
| 61 | MM | **G-CSF+plerixafor** | 22 | 2 | **8.32** |
| | | G-CSF+chemotherapy | 19 | 2 | 0.66 |
| 46 | MM | **G-CSF+plerixafor** | 40 | 1 | **8.74** |
| | | G-CSF+chemotherapy | 0 | | |
| 42 | MM | **G-CSF+plerixafor** | 300 | 1 | **43.08** |

**1b.** Summary of the results for these 20 patients with each method of stem cell mobilization

| | Median of circulating CD34+ cells µ/L | Median of collected CD34+ cells $10^6$/kg |
|---|---|---|
| **G-CSF+chemotherapy** | 4.25 | 0.35 |
| **G-CSF** | 4 | 0,24 |
| **G-CSF+plerixafor** | **35.5** | **6.05** |

[P705] **Figure 2.** Algorithm decisional on the use of G-CSF+Plerixafor in first mobilization procedure




quences on transplantation time point and hospitalisation costs.
Objectives We aimed at establishing the efficiency of Plerixafor administration in 'poor mobilizers' multiple myeloma (MM) and lymphoma patients.
Methods: In a unicentric retrospective study data records on 7 patients enrolled between 2009-2011 were analyzed. Pathological background consisted of MM (n=4) and non-Hodgkin lymphoma (NHL) (n=3) with a mean age of 43.85 yr (range 23-61) and a sex ratio of 1.33:1 (male:female). Two NHL patients received previous radiotherapy and 2 of the MM cases were treated with Alkeran based regimens before chemomobilization. All patients received chemotherapy followed by administration of G-CSF (10 ug/kg/day) and Plerixafor was added starting with day 6. Apheresis procedures were performed on Cobe Spectra-MNC protocol.
Results: Plerixafor was administrated in 4 patients with failure to achieve an absolute count of CD34+ cells in peripheral blood >10/uL while in 3 cases, the prior apheresis products contained insufficient CD34+ cells (<2.5x10$^6$/kg). Standard dose for Plerixafor was 0.24 mg/kg, with an average number of 2.4±0.69

doses/cycle of chemomobilization, while the average number of doses/patient was 3.42±1.27 with the following distribution:5 doses-2 patient, 4 doses-1 case, 3 doses-2 patients and 2 doses-2 subjects. In 3 patients chemomobilization with Plerixafor was applied 2 times, while the remaining subjects received only 1 chemomobilization cycle. We performed 14 apheresis procedures (AP), more than $2.5x10^6$ CD34+ cells/kg being collected after 2 AP in 3 patients, 1 AP in 2 cases and 4 AP in 1 case; in 1 patient the established target of CD34+ cells/kg was not achieved after 2 mobilization cycles with Plerixafor. After Plerixafor administration the absolute value of CD34+ cells increased 2 folds in 3 subjects, 4 folds in 2 patients and more than 4 folds in 2 patients. Five patients (3-MM; 2-NHL) underwent autologous hematopoietic stem cell transplantation and mean engraftment day for granulocytes and platelets was 16.25±2.21 respectively 22±0.81; one patient ceased 7 days after stem cells infusion.
Conclusion: Plerixafor combined with chemomobilization was significantly more effective in collecting the target number of CD34+ cells in 6 subjects from our population of 'poor mobilizers' with a good impact on engraftment time points.

**P707**
**NK alloreactivity is reduced by GCSF but enhanced by plerixafor**
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Introduction: Granulocyte colony-stimulating factor (GCSF) and under certain circumstances Plerixafor, an antagonist of the CXCR4-SDF 1a axis, are used for stem cell (SC) mobilization in autologous or allogeneic stem cell transplantation (SCT). Earlier studies indicated a down-regulating effect of GCSF on T cell activity. Here we asked for the immune modulating capability of these agents towards NK cell effector functions.
Methods: Cytotoxicity and cytokine release of NK cells were studied in peripheral SC donors (N = 20) before and 5 days after GCSF administration (in-vivo stimulation). In addition, NK cell functions were analysed after culturing of peripheral blood cells from healthy blood donors (N =12) in presence and absence of GCSF and Plerixafor (in-vitro stimulation). NK cells were isolated by negative selection and their functions were studied using the HLA-E*01:03 transfected K562 cells and the parental HLA class I negative K562 cell line as target cells. Cytotoxicity was determined by flow cytometry after propidium iodide staining. IFN-gamma and sFasL release were detected in culture medium by ELISA. Receptor expression and adhesion molecule profiling were performed for CD3, CD56, CD45, CD94, NKG2A, NKG2C, CD2, CD18, CD50, CD58 and CD62L.
Results: The NK cells showed a reduced cytotoxicity towards K562/K562:HLA-E*01:03 target cells after in-vivo or in-vitro GCSF stimulation compared to not-treated NK cells ($p<0.05$). The proportion of HLA-E specific receptors (CD94+NKG2A+) is reduced by GCSF ($p=0.011$). GCSF reduces the IFN gamma and sFasL release of NK cells in-vivo ($p=0.004$) and in-vitro ($p=0.03$). The percentage of CD56bright cells on total NK cells and the expression of the CD2, CD50 and CD62L on CD56bright NK cells are significantly decreased by GCSF stimulation ($p<0.01$). Contrary to GCSF, Plerixafor leads to enhanced NK cytotoxicity and sFasL release ($p<0.05$) and tentatively to increased expression of the adhesion molecules CD2, CD18 and CD58 on CD56dim cells. The IFN gamma release seems not to be affected by Plerixafor.
Conclusion: To the best of our knowledge this study is the first one showing that GCSF impairs NK functions, whereas Plerixafor leads to an enhanced NK alloreactivity. Thus, Plerixafor might lead to a more beneficial immune reconstitution – which may be especially relevant in the haploidentical SCT setting.

**P708**
**Plerixafor and G-CSF combination leads to excellent PBSC mobilisation and collection in Jehovah's Witnesses patients: experience at the European Institute of Oncology**
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Background: G-CSF with or without chemotherapy is the most common strategy for PBSC mobilization. Recently biosimilar forms of G-CSF have been approved for the same indication as the reference filgrastim. Plerixafor, a CXCR4 antagonist, has been shown to increase the number of CD34+ cells in peripheral blood in pts with lymphoma and multiple myeloma (MM) and to rescue pts unable to mobilize with traditional approach with a good safety profile. Jehovah's Witnesses (JW) form a religious group of about six million members worldwide that, for religious reasons, refuse transfusion of any major blood products; the use of full dose of mobilizing chemotherapy is therefore not recommended in these pts. A mobilization approach that allows an adequate PBSC collection and avoids risks of myelosuppression is strongly preferred in JW pts.
Aim: To assess the efficacy of the combination of originator filgrastim or Filgrastim XM02 (Tevagastrim) and plerixafor in achieving the optimal required dose of CD34+ cells for ASCT ($\geq 4x10^6$ CD34+ cells/Kg) in JW pts affected by MM and non-Hodgkin lymphoma (NHL).
Patients and Methods: We retrospectively analyzed mobilization procedures of 7 JW pts, median age 56 years (36-68), affected by MM (#6) and NHL (#1), scheduled to receive an ASCT. Six out of seven pts had never been mobilized before, while 1 had already received a previous ASCT. Tevagrastim (#4) or originator filgrastim (#3) was self-administered at 10ug/kg/die for 3 days; on day 4 plerixafor (0.24 mg/kg) was administered 12 hours before the apheresis procedure.
Results: The median number of circulating CD34+ cells following plerixafor administration was 91/ul (13-138). Median number of CD34+/kg cells collected was $5.6x10^6$/kg (2.4-8.4) by 1 procedure in 5 pts and 2 in the others; the only patient who collected $\leq 4x10^6$ cells/kg was the who had already received a previous ASCT; 4/6 pts affected by MM collected the amount of CD34+ cells/kg needed for a tandem transplant ($\geq 4x10^6$ cells/kg) by 1 procedure, with a median number of $6.7x10^6$ cells/kg (4.1-8.4). No major side effects were reported. All the pts underwent stem cell transplantation with Melphalan (140 mg/sqm) and engrafted.
Conclusions: Our data show that originator filgrastim or Tevagrastim in combination with plerixafor is a feasible and safe approach in JW pts and allows collection of adequate number of PBSC needed for ASCT avoiding chemotherapy related side effects.

**P709**
**Plerixafor in combination with originator or biosimilar XM02-G-CSF as first-line peripheral blood stem cell mobilisation strategy in patients with lymphomas and multiple myeloma candidate to ASCT: a single-centre experience**
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Plerixafor, a CXCR4 chemokine antagonist, has shown to increase the number of circulating CD34+ cells in cancer patients alone or in combination with G-CSF and to be able to rescue patients (pts) unable to mobilize with traditional approach. Recently, several forms of biosimilar nonglycosylated recombinant human G-CSF have been approved for the same indications as the reference filgrastim on the basis of comparable quality, efficacy, and safety. Biosimilars also provide a more cost-effective strategy and their use in clinical

setting may provide cost savings. We therefore used plerixafor in combination with originator G-CSF or biosimilar version of G-CSF (Tevagrastim) as first line mobilization strategy in pts with Lymphoma or Multiple Myeloma (MM) scheduled to receive an ASCT. The primary endpoint was the proportion of pts mobilizing ≥20 cells/µl on day 5 and collecting ≥2.0 x $10^6$ CD34+ cells/kg on the same day.
From April 2010 to November 2011, 28 pts, median age 57 yrs (17-68), affected by Non-Hodgkin Lymphoma (#12), Hodgkin Disease (#3) and MM (#13), received a combination of G-CSF (#7) or Tevagrastim (#21) and plerixafor as first line strategy for peripheral blood stem cells (PBSC) mobilization; median lines of previous chemotherapy was 2 (1-6). Filgrastim or Tevagrastim was self-administered (10ug/kg/die) for 3 days; on day 4 plerixafor was injected (0.24mg/kg) 12 hours before the scheduled apheresis. On day 5 median number of circulating CD34+ cells was 52/ul (10-138) in 24 pts; 4/28 pts (14%) were unable to mobilize ≥20 cells/µl on day 5 and required a second plerixafor administration. All 28 patients were able to collect an adequate number of CD34+ cells necessary for the transplantation procedure with a median number of 4x$10^6$ (2.2-10.6) CD34+cells/kg in a median number of 1 procedure (1-2); 19/28 (68%) pts harvested the required CD34+ dose on day 5. For pts with MM, 7/13 (53%) collected ≥4×$10^6$ CD34+ cells/kg in a single procedure. In terms of mobilization, no difference was observed with the use of G-CSF or biosimilar Tevagastrim and no major side effect was reported either with G-CSF or biosimilar Tevagastrim. The combination of G-CSF or tevagrastim and plerixafor is safe and effective in mobilizing PBSC and allows a collection of a more than adequate number of cells in most of the pts in a maximum of 2 apheresis procedure, even in pts with MM who need to collect a double amount of cells for a tandem transplant.

**P710**
**Stem cell mobilisation for autologous transplantation: outcomes and rationalising the indications for using plerixafor**
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Reported rates of stem cell mobilisation failure prior to autologous stem cell transplantation (ASCT) are variable (5-40%) according to the study population, target yield and mobilisation protocol. There has been recent interest in using Plerixafor to enhance mobilisation, although a lack of consensus about indications for use. We report the outcome of stem cell mobilisation at a single centre using a uniform treatment strategy. 154 sequential patients treated during 2010-11 were included. Patient demographics & treatment are shown in table 1. Target yield was >2x$10^6$ CD34+ cells/kg in ≤3 harvests; >4x$10^6$ CD34+ cells/kg were collected if possible for myeloma patients to allow a potential second transplant. Harvesting was attempted if circulating CD34+ count was >10x$10^6$/l.
94.2% (145/154) of patients mobilised >2x$10^6$ CD34+ cells/kg (median 4.73x$10^6$ range 0-38.9) after a total of 201 harvests (1-3 per patient). 67% (32/48) of myeloma patients achieved >4x$10^6$ CD34+ cells/kg. Median circulating CD34+ cell count on day 1 of first harvest was 57.9x$10^6$/l (range 0-990); 10/154 patients had a count of <10 x$10^6$/l and harvesting was not attempted. Circulating CD34+ cell count correlated with yield (Spearman's rho 0.85). The optimal predictive CD34+ count for successful harvest was >15x$10^6$/l (ROC analysis: sensitivity 97%, specificity 83%).
14 patients required re-mobilisation (total 18 procedures) utilising a Plerixafor based regimen (6/18) or GCSF±chemotherapy (12/18). 67% achieved the target yield using Plerixafor *vs* 33% where Plerixafor was not used (p=NS). 61% (94/154) of patients proceeded to ASCT; most common reasons not to proceed were progressive disease, allogeneic transplant or patient choice. All transplanted patients engrafted with median time to neutrophil (>0.5x$10^9$/l) and platelet (>20x$10^9$/l) recovery of 12 days (range 9-20) and 13 days (range 9-62) respectively.
The majority of patients achieved yield >2x$10^6$ CD34+ cells/kg and prompt engraftment following ASCT. Plerixafor could potentially facilitate a higher yield although this is not routine practice in the UK. CD34+ cell yield correlated strongly with circulating count on the day of harvest and Plerixafor should be considered if predictive CD34+ count is low; our results suggest an optimal cutoff of <15x$10^6$/l. This may obviate the need for re-mobilisation (required in 9.1% of the cohort) although further studies are needed to confirm the efficacy & cost-effectiveness of this approach.

Table 1: Patient demographics and procedures

| | |
|---|---|
| **Sex** | |
| Male | 105 |
| Female | 49 |
| **Median Age** | 50 (16-71) |
| **Diagnosis** | |
| Multiple Myeloma | 48 |
| Non-Hodgkin Lymphoma | 69 |
| Hodgkin Lymphoma | 24 |
| Other | 13 |
| **Median Prior Therapies** | |
| Multiple Myeloma | 1 (1-3) |
| Lymphoma | 2 (1-4) |
| **Mobilisation (Myeloma)*** | |
| Cyclophosphamide (1.5g/$m^2$) | 41 |
| GCSF† | 2 |
| ESHAP/DHAP | 5 |
| **Mobilisation (Lymphoma)*** | |
| Cyclophosphamide (1.5g/$m^2$) | 8 |
| GCSF† | 1 |
| ESHAP/DHAP ± Rituximab | 53 |
| CHOP ± Rituximab | 12 |
| Other salvage chemotherapy | 19 |

* Refers to first mobilisation regimen used
† GCSF (Granulocyte Colony Stimulating Factor) was used for all procedures, initiated day+1 following mobilisation regimen. Doses were 300/480 mcg per day (Filgrastim) or 263/526 mcg per day (Lenograstim); the higher doses were used for patients with body surface area >1.8$m^2$

**P711**
**Successful use of plerixafor in combination with G-CSF to achieve mega-doses of progenitors from healthy donors for haploidentical T-depleted stem cell transplantation**
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Low numbers of infused progenitors is a risk factor for primary graft failure, especially for patients receiving a T-depleted haploidentical stem cell transplantation (haplo-SCT), where mega-doses are required for engraftment. Plerixafor (Mozobil®) causes mobilization by disrupting the SDF-1/CXCR4 interaction.
At our Institute, 2 healthy donors for patients candidate to haplo-SCT did not achieve the expected target of 7x$10^6$ CD34+/kg recipient with conventional mobilization (G-CSF 10 mcg/kg daily) and were successfully mobilized with plerixafor.
A 61 yrs old male (86kg) affected by high risk acute myeloid leukemia (AML) was candidate to a T-depleted haplo-SCT. On day 4 of G-CSF mobilization, the 37 yrs old female donor (50kg) had 33/mcL circulating CD34+(WBC42x$10^3$/mcL) resulting in a harvest of 1.27x$10^6$CD34+/kg recipient (0.9x$10^6$CD34+/kg post CliniMACS Milteni positive immunoselection). Following informed consent for off-label use, the donor received a single

subcutaneous (sc) injection of 24 mgs plerixafor (0.48 mg/kg) in combination with G-CSF with no side effects; 8 hrs later: circulating CD34+: 134/mcL (WBC 64x$10^3$/mcL), harvest: 7.04x$10^6$ CD34/Kg recipient (post selection 5.64x$10^6$ CD34+/Kg, efficiency 80%, 0.5x$10^4$ CD3+/Kg). Engraftment: neutrophil >500/mcL day 20, >1000/mcL day 24; platelets >20,000/mcL day 18, >50,000/mcL day 21.
A 62 yrs old female (55 kg) affected by high risk AML was candidate to a T-depleted haplo-SCT. On day 5 and 6 of G-CSF mobilization, the 58 yrs old male donor (70kg) had 29 and 35/mcL circulating CD34+ (WBC36 and 37x$10^3$/mcL) resulting in a harvest of 3.93 and 3.42x$10^6$CD34+/kg recipient (total dose post immunoselection 4.59x$10^6$CD34+/kg, efficiency 62%, 0.39x10e4 CD3+/kg). To increase the cell dose, following informed consent, the donor received a sc injection of 24 mgs plerixafor (0.34 mg/kg) in combination with G-CSF with no side effects; 8 hrs later: circulating CD34+: 77/mcL(WBC71x$10^3$/mcL); harvest 8.2x$10^6$CD34+/Kg recipient (post selection 4.9x$10^6$CD34+/kg, efficiency 59%, 0.19x$10^4$CD3+/Kg). The patient received a total dose of: 9.49x10e6CD34+/kg, 0.58x10e4CD3+/kg. Engraftment: neutrophil >500/mcL day 22, >1000/mcL day 23; platelet >20,000/mcL day 14, >50,000/mcL day 19.
In conclusion, plerixafor should be considered for family donors failing mobilization or with an unfavourable weight ratio when mega-doses of progenitors are needed. In addition, plerixafor as a mobilizing agent does not interfere with the CD34+ immunoselection.

**P712**
**Mobilisation of haematopoietic stem cells by plerixafor alone in children: a sequential Bayesian trial**
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Background: The rapid kinetics of hematopoietic stem cells induced by plerixafor (Mozobil®, Genzyme) could be valuable in children. We conducted a prospective trial to determine whether a one-day mobilization by plerixafor alone was sufficiently efficient in children with cancer.
Methods: Children with solid malignancies were consecutively recruited for this phase IIA, Bayesian single-centre prospective trial. Mobilization consisted of one subcutaneous injection of 240 µg/kg body weight plerixafor at 8 a.m. (h0). Collection by apheresis began at h5 provided the level of CD34+ exceeded 10x$10^6$/L. Our main evaluation criterion was the percentage of children in whom at least 5x$10^6$ CD34+/kg could be collected during the first apheresis.
Results: No patient met the success criterion, so the trial ceased after five patients on set stop criteria. All the patients reached the threshold value of 10x$10^6$ CD34+cells/L after injection, and apheresis could be performed in all cases. The median number of CD34+ cells collected was 1.62x$10^6$/kg BW [0.47-3.5]. Three out of the five patients yielded >1.5x$10^6$ CD34+/kg BW. The peak levels of CD34+cells ranged from 11x$10^6$/L to 44x$10^6$/L and were reached in 4 to 6 hours. No side-effects were observed.
Conclusion: A one-day mobilization regimen consisting of one injection of 240 µg/kg plerixafor alone with collection of HSC starting at h3 was not efficient enough to collect a complete graft, but may be an attractive option for completing an insufficient graft.

**P713**
**Mozobil (plerixafor) - decision for "poor mobilisers" in autologous stem cell transplantation**
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Poor peripheral blood stem cell (PBSC) mobilization has been reported as an obstacle to autologous stem cell transplant (ASCT). It is known that 30% of patients, planed to be transplanted are "poor mobilizers". Plerixafor (AMD3100) has been recently approved as a mobilization agent for PBSC in the setting of ASCT.
Methods: 10 patients was treated with Plerixafor in our BMT Unit (6 with multiple myeloma and 4 with Non-Hodgkin's lymphoma). Patients average age of 50.8 years (between 30 to 64 years) and male to female ratio of 3:7. All patients received Mozobil in combination with G-SCF in defined schedule.
Results: For all treated with Plerixafor patients, the number of CD34+ in previous collections was under the limit for successful transplantation. After Mozobil application the number of CD34+ increased over 20 cells/µl and suffiecient harvest was obtained. For all patients mobilisations were successful without serious complications. The number of apheresis was 17, median number of Mozobil application was 1.7 vials per patient, and median number of collected CD34+ was 3.07 x$10^6$ kg. All patients were successfully transplanted. The median time to neutrophil and platelets engraftment was at 12 days and 12.9 days, respectively.
Conclusions: For all "poor mobilizers" adequate transplant CD34+ cell dose was obtained by combination of Plerixafor with G-CSF. Patients mobilized with Plerixafor have the same post transplant recovery and long-term outcome. There are pharmacoeconomic's advanteges in the long term prices of ASCT. Our data suggest that Plerixafor (Mozobil) administered with standard mobilisation cytokine (G-CSF) could be effective to patients previously failed G-CSF PBSC mobilisation attempts.

**P714**
**Up-front use of plerixafor combined with chemotherapy and G-CSF in hard-to-mobilise lymphoma patients gives a satisfactory yield of CD34 cells within 1-2 days of apheresis and shows a sustained engraftment following high-dose therapy**
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In our hospital mobilization of autologous hematopoietic stem cells in lymphoma patients is done by standard induction chemotherapy and G-CSF. To obtain a quick and sustained engraftment after high dose therapy, a minimal number of 2x$10^6$ CD34+ cells/kg are desirable. However, in 15-20% of the lymphoma patients characterized as poor mobilizers, insufficient numbers of CD34+ cells are harvested, either prompting remobilization, or can not be offered high dose therapy even if it could be curative. Recently, new mobilizing agents including plerixafor, a CXCR4 antagonist, have been developed. Our own experiences have shown that when a low concentration of CD34+ cells (4-10 cells/µL) and a total white cell count >10x$10^9$/L is seen in patients given chemotherapy and G-CSF they are hard to mobilize and addition of plerixafor should be considered.
In the present study, we have mobilized and harvested 12 poor mobilizing lymphoma patients. Two patients were remobilized with G-CSF and plerixafor, whereas 10 patients were given plerixafor up-front in the primary mobilization attempt in addition to chemotherapy and G-CSF. The patients were then followed after reinfusion of autologous stem cells with regard to short and long term engraftment.
All patients were successfully harvested (median: 4.2x$10^6$ CD34+cells/kg; range: 1.8-7.2x$10^6$ CD34 cells/kg).Then, all patients received high dose therapy followed by autologous stem cell support. Time to short term engraftment, defined by neutrophils >0.5x$10^9$/L and thrombocytes >20x$10^9$/L were 10.5 days (median; range 9-16) and 14.5 days (median; range 11-100). As an estimate on long term engraftment we also examined the thrombocyte levels at day 100 after reinfusion. The median level was 114x$10^9$/L (range 27-398). Notably, the patient with a thrombocyte value of 27, received only 1.8x$10^6$ CD34+ cells/kg, but had a satisfactory number of CFU-GM/kg

at the time of infusion. He has later developed a pancytopenia, and further examination has revealed myelodysplasia in this patient. For comparison, we examined the thrombocyte levels of 12 consecutive lymphoma patients receiving high dose therapy with autologous stem cell support after a normal stem cell mobilization, and observed day 100 thrombocyte levels of 213x10$^9$/L (median; range 95-338).
We conclude that our recommendation to use up-front plerixafor in hard to mobilize lymphoma patients is successful in 10 out of 10 patients. Furthermore, only 1 or 2 days of apheresis was needed.

**P715**
**Plerixafor pus filgrastim biosimilar can mobilise CD34+ progenitor cells from multiple myeloma and lymphoma patients failing previous mobilisation attempts: an Italian multicentre study**
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Plerixafor is an inhibitor of CXCR4 receptor /SDF-1 binding used in combination with G-CSF for mobilization of autologous peripheral blood hematopoietic progenitor cells (auto-PBSCT). In this study, we collected data from 211 patients with various hematological disorders (multiple myeloma, MM, n 81, non Hodgkin lymphoma, n. 105, Hodgkin lymphoma, n 25). 144 patients were considered proven poor mobilizer, since they have had a previous mobilization failure, and 65 were classified as predicted poor mobilizer according to GITMO criteria (BMT, 2011, May 30:1-10). All patients received plerixafor (Mozobil-Genzyme-Sanofi) plus G-CSF with or without chemotherapy in 23 Italian centres. A total of 140 patients (68%) collected ≥ 2 x 10$^6$ CD34+ cells/kg. The collection yield was significantly higher in MM patients (82%) than in HL (70%) and NHL (57%). Eighteen patients were mobilised using G-CSF biosimilar (Tevagrastim-Teva) plus Plerixafor in proven poor mobilizer patients; 15 of them mobilized ≥ 2 x 10$^6$/kg CD34+ cells. Overall, 83% of patients reached ≥ 20 CD34+ cells/uL with plerixafor plus filgrastim biosimilar, after a median of 3.6 fold increase in CD34+ cells/uL from day 4 to day 5. No side effects were observed in the cases examined. Eighty % of the patient subgroup having ≥ 2 x 10$^6$/kg CD34+ cells, were transplanted with CD34+ cells mobilised with the combination of G-CSF biosimilar and plerixafor. Short term recovery of platelet and neutrophil counts after auto-PBSCT, and safety profile post-PBSCT were found to be comparable with that of the patient subgroup receiving plerixafor plus originator filgrastim. These data show the combination of biosimilar filgrastim and plerixafor is effective and provides a non-toxic approach to mobilise stem cells.

**P716**
**Pre-emptive plerixafor use after chemomobilisation: a single-centre experience of 40 patients**
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Background: Stem cell mobilization is difficult in a significant proportion of patients intended for ASCT. Recently plerixafor, a CXCR4 antagonist, has become for clinical use to facilitate more effective mobilization of blood stem cells in patients who appear to mobilize poorly. We have evaluated our experience on plerixafor use added to chemomobilization.
Patients and Methods: Between VIII/2009 and IX/2011 altogether 40 patients have received plerixafor pre-emptively in our hematology ward. There were 15 females and 25 males (median age of 57 years, range 20-68 years). Twenty-nine patients had non-Hodgkin lymphoma, seven patients myeloma and four patients Hodgkin lymphoma. The indications to use plerixafor included low blood CD34+ (B-CD34+) counts (median 4 x 10$^6$/L) at the time of marrow recovery (n=30), poor prior collection (<1 x 10$^6$/kg CD34+ cells) (n=7) and/or low/decreasing B-CD34+ counts (n=3). The median number of plerixafor injections was 1 (1-4).
Results: The median fold increase in B-CD34+ cell counts to the next morning after the first plerixafor dose was 4. Stem cell collections were started altogether in 34 patients (81%). The median number of CD34+ cells collected was 2.9 x 10$^6$/kg (0.5-7.8 x 10$^6$/kg) with median of two aphereses. The minimum collection target (≥2 x 10$^6$/kg CD34+ cells) was achieved in 31 patients (78%). In six patients the maximum B-CD34+ cell counts were only 1-6 x 10$^6$/L and no aphereses were attempted. The nine patients who failed to reach the minimum collection target were comparable to successful mobilizers in regard to gender (males 67% *vs.* 61) %), age (59 *vs.* 57 years) or diagnosis (myeloma *vs.* lymphoma) (29% *vs.* 24%). Patients who failed the minimum collection target had lower B-CD34+ counts before plerixafor (median <1 *vs.* 7 x 10$^6$/L, p<0.001) as well after the first plerixafor dose (2 *vs.* 41 x 10$^6$/L, p<0.001). If those patients with suboptimal collections are excluded, all patients who failed the collection had the peak B-CD34+ counts ≤10 x 10$^6$/L after 1-4 plerixafor injections.
Conclusions: Plerixafor in efficient in the majority of chemomobilized patients mobilizing poorly or having poor collections. Baseline characteristics are not different in those failing to mobilize even with the addition of plerixafor, except for lower B-CD34+ counts.

**P717**
**Kinetics of blood CD34+ cells after chemotherapy plus G-CSF in poor mobilisers: implications for pre-emptive plerixafor use**
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Background: Mobilization and collection of blood stem cells is difficult in a significant proportion of patients intended for autologous stem cell transplantation. Recently a CXCXR4 antagonist, plerixafor, has been approved for clinical use in patients who appear to mobilize poorly but algorithms for optimal use of this expensive drug are lacking. We have therefore analyzed kinetics of blood CD34+ cells (B-CD34+) mobilization in relation to recovery of white blood cell (WBC) counts after chemomobilization in poor and standard mobilizers to find out in which circumstances to use plerixafor.
Patients and Methods: Altogether 390 chemomobilized patients were included into this retrospective analysis. We classified patients with maximum B-CD34+ cell counts ≤5 x 10$^6$/L as very poor mobilizers, those with maximum B-CD34+ cell counts 6-10x10$^6$/L as poor mobilizers, and patients with collections ≥2 x 10$^6$/kg CD34+ cell with maximum of three aphereses as standard mobilizers, respectively.
Results: There were 43 patients (11%) fulfilling the criteria for poor or inadequate mobilization. The proportion was highest in patients with non-Hodgkin lymphoma (17%) followed by Hodgkin lymphoma (9 %) and myeloma (3%). In these patients B-CD34+ counts rarely rose after recovery of WBC > 5-10x10$^9$/L unlike the situation in standard mobilizers. Considering all patients having B-CD34+ peak ≤ 10 x 10$^6$/L as candidates for preemptive plerixafor use, four algorithms were constructed: model A WBC > 5 x 10$^9$/L and B-CD34+ ≤ 5 x 10$^6$/L, model B WBC > 5

x $10^9$/L and B-CD34+ ≤ 10 x $10^6$/L, model C WBC > 10 x $10^9$/L and B-CD34+ ≤5 x $10^6$/L, and model D WBC > 10 x $10^9$/L and B-CD34+ ≤ 10 x $10^6$/L. Of these models B and D were the best (sensitivity 0.97 and 1.0, respectively) but model B was more widely applicable in poor mobilizers in this series.
Conclusions: Based on this retrospective study, plerixafor should be considered in chemomobilized patients if WBC is rising and > 5 x $10^9$/L and B-CD34+ cell count remain ≤10 x $10^6$/L. This simple model needs a prospective validation.

**P718**

**Prevalence, predictive factors of poor mobilisation and peak CD34+ cell count in peripheral blood to guide pre-emptive or immediate salvage mobilisation in poor mobilisers with lymphoma or multiple myeloma**

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Background and Objective: Mobilization failure of peripheral blood stem cells (PBSC) constitutes a frequent reason for not performing autologous stem cell transplantation (SCT) in patients with lymphoma or multiple myeloma (MM). Pre-emptive or immediate salvage mobilization with plerixafor through early identification of poor mobilizers could avoid repeated attempts of mobilization, decreasing resource utilization, morbidity and delaying of SCT.
Patients and Methods: Data from first mobilization of 287 patients with lymphoma or MM consecutively referred to a transplant unit for autologous SCT between 2000 and 2010 were collected. Poor mobilization was defined as the collection of CD34+ cell dose of less than 2x$10^6$/Kg BW.
Results: Median of age was 54 years (range 9-70) and 180 (63%) were males. Diagnosis was multiple myeloma (MM) in 133 cases, non-Hodgkin's lymphoma in 112, and Hodgkin's lymphoma in 42. A total of 34 patients has received 3 or more therapy lines, including alkylating agents in 225 (78 %), purine analogues in 30 (11%) and radiotherapy in 47 (16%). At the time of mobilization 103 (37%) patients were in complete remission (CR) whereas 184 had active disease. Mobilization regimen consisted of G-CSF in 245 patients (85%) and chemotherapy followed by G-CSF (C + G-CSF) in 42 (15%). Poor mobilization was observed in 73 patients (25%) (36% in lymphomas and 13% in MM), without differences according to mobilization schedule. At the multivariate analysis, diagnosis, previous therapy with purine analogues, prior chemotherapy lines ≥ 3 and low platelet count were predictive factors for poor mobilization. A CD34+ cell count in peripheral blood (PB) higher than 13.8 CD34 cells /µL was enough to ensure adequate PBSC collect (≥2x106 CD34+ cells/Kg BW), with high sensitivity (91%) and specificity (91%).
Conclusions: The prevalence of poor mobilization was high. The diagnosis of lymphoma (compared to MM), therapy with purine analogs or multiple chemotherapy regimens and low platelet count were associated with mobilization failure. A peak CD34+ cell count in PB <13.8/µL was associated with harvest of <2x$10^6$ CD34+ cells/kg BW and can be useful to guide pre-emptive or immediate plerixafor.
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**P719**

**Plerixafor for autologous peripheral blood stem cell mobilisation in patients previously treated with fludarabine or lenalidomide**

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High dose chemotherapy followed by autologous hematopoietic stem cell transplantation (HSCT) is an effective treatment for non-Hodgkin lymphoma (NHL) and multiple myeloma (MM). At present, G-CSF-mobilized peripheral blood stem cells (PBSCs) are the preferred stem cell source for autologous HSCT. Fludarabine and lenalidomide are essential drugs in the front line treatment of NHL and MM respectively. Data suggests that fludarabine and lenalidomide may have a deleterious effect on stem cell mobilization. Prior to the drug approval in Europe, a plerixafor compassionate use program (CUP) was available from July 2008 to August 2010 to provide access to the drug for patients with MM or lymphoma who had previously failed a mobilization attempt, and who were not eligible for another plerixafor trial.
In the European CUP, 48 patients (median age 57 years; range, 36-69), previously treated with fludarabine (median 5 cycles; range, 1-7 cycles) were given plerixafor plus G-CSF for remobilization following a primary mobilisation attempt. All 48 patients had a diagnosis of NHL. The overall median number of CD34+ cells collected was 2.3×$10^6$ /Kg (range, 0.3-13.4). The minimum required number of CD34+ cells (≥2.0×$10^6$ per kg) was collected from 58% of patients, 3 patients (6%) collected ≥5.0×$10^6$ CD34+ cells. The collection target of 2.0×$10^6$/Kg was reached in a median of 2 apheresis sessions (range, 1-3). Thirty-five patients (median age 57 years; range, 34-66), previously treated with lenalidomide (median 5 cycles; range, 1-10 cycles) were given plerixafor plus G-CSF for remobilization. All patients the 35 patients had MM. The overall median number of CD34+ cells collected was 3.4×$10^6$/Kg (range, 1.1-14.8). The minimum required number of CD34+ cells (≥2.0×$10^6$ per kg) was collected from 69% of patients, including 12 patients (34%) who were able to collect ≥5.0×$10^6$ cells/Kg. Both targets were reached with a median of 2 apheresis sessions (range, 1-4).
In conclusion, salvage mobilization with plerixafor plus G-CSF is successful in the majority of patients with MM previously treated with lenalidomide. In fludarabine-exposed patients, only 58% of patients will achieve successful salvage mobilization with plerixafor plus G-CSF, suggesting the need for large prospective studies evaluating the efficacy of plerixafor for frontline mobilization in this patients.

**P720**

**A retrospective audit of autologous stem cell collection to guide the use of 'just-in-time' plerixafor**

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Stem cell transplantation is an integral part of the management of several haematological disorders and autologous

transplantation is part of standard of care in myeloma and relapsed lymphomas. Historically around 30% of all patients fail to mobilise sufficient CD34+ cells, thus are potentially denied access to this standard of care. Plerixafor, a CXCR4 antagonist was approved by the Scottish Medicines Consortium in January 2010 within its licensed indication for patients with lymphoma and myeloma whose stem cells mobilise poorly. Mobilisation with GCSF and Plerixafor costs around 16 times more than with cyclophosphamide and GCSF. Therefore we need to identify the most cost-effective way of using Plerixafor.

From our local apheresis database, we identified all stem cell collection attempts and the dose of CD34+ cells collected, from January 2000 to July 2011.

Over this 11½ year period, stem cell collection was attempted on 389 occasions from 371 individuals. 30 of these were healthy donors who readily mobilised sufficient numbers of stem cells. Locally we aim for a minimum autologous collection of $3.5x10^6$ CD34+ cells/Kg body weight. 100 collections (25.7%) failed to reach this threshold. 13.6% failed to mobilise our minimum accepted threshold for autologous stem cell transplantation of $2x10^6$/Kg CD34+ cells. 18 patients were mobilised with Plerixafor. Most had failed previous mobilisation attempts with standard regimens. 3 were felt to be at high risk of mobilisation failure, therefore received Plerixafor up front. CD34+ doses collected ranged from $1.01–22.4x10^6$/Kg and 16 patients (89%) achieved the minimum stem cell dose required of $>2x10^6$/Kg.

There was a linear relationship between peripheral blood CD34 counts and CD34+ dose collected (correlation coefficient 0.74). 75% of patients with peak peripheral blood CD34 counts of $<15x10^6$/L, failed to mobilise $2x10^6$/Kg CD34+ cells. Based on these findings we have written a local Standard Operating Procedure to guide the use of ‚immediate rescue' or ‚just in time' Plerixafor. GCSF, 5ug/Kg is commenced on day +5 after completion of mobilising chemotherapy. Peripheral blood CD34

**[P720]**

**Relationship between peripheral blood CD34 count and CD34+ cell dose collected at apheresis**

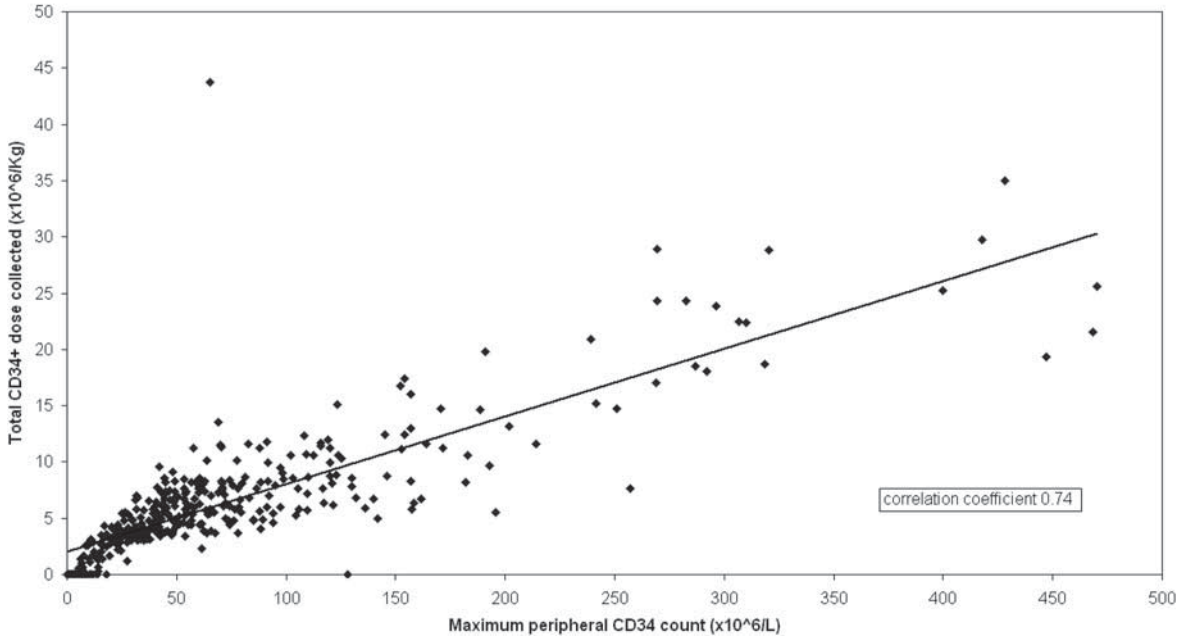


Table 1. Outcome of poor mobilisers, defined as peripheral blood CD34 count <20x10^6/l

| Maximum peripheral CD34 count (x10^6/l) | Total number of patients | Number of patients undergoing apheresis | Total CD34+ collection >2.0* |
|---|---|---|---|
| <10 | 38 | 10 | 2 (5.3%) |
| 10-14.5 | 16 | 12 | 4 (25%) |
| 15-19.5 | 13 | 12 | 9 (69.2%) |

*units $x10^6$CD34+cells/kg body weight

counts begin on day +10. We aim for a CD34 count of $20x10^6$/L prior to commencing apheresis. The addition of Plerixafor is recommended if the CD34 count fails to reach $15x10^6$/L. Sepsis must be excluded and the dose of GCSF increased to 10ug/Kg. This targets the population most likely to benefit while minimising cost.

**P721**

**Mobilisation of peripheral blood stem cells for autologous transplantation using plerixafor (Mozobil) and G-CSF – single-centre experience**

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High-dose chemotherapy followed by autologous peripheral blood stem cell (PBSC) support is a salvage treatment option for patients with relapsed or refractory hematological malignancies and solid tumors. Plerixafor (Mozobil) is a selective and reversible CXCR4 antagonist and interrupts interaction of CXCR4 with SDF-1, thus resulting in rapid mobilization of stem cells in peripheral blood for collection and subsequent autologous transplantation.
We present our experience with mobilization of PBSC using Plerixafor plus G-CSF.
Between March 2010 and May 2011 eight patients with different cancers were mobilized with Plerixafor plus G-CSF and seven of them later were transplanted with autologous graft in our center. The median age was 27, 5 years (7-44). Three of the patients were children. Four patients were with Hodgkin disease (two children and two adults), one with NHL, one with multiple myeloma and two with germ cells tumors (1 child and 1 adult). Five patients had previously one and three patients - two unsuccessful mobilizations. Plerixafor was obtained on a compassionate basis from Genzyme Bulgaria. In six patients mobilization not including chemotherapy was started during patient's hematological steady state with a 4-day subcutaneous administration of G-CSF at 10 μg/kg once daily. In the evening of the day 4 and in some patients on the day 5 and 6, at 11 h before apheresis, Plerixafor at 240 μg/kg was subcutaneously administered. G-CSF was given on day 5, 6 and 7 at 1 h before apheresis. In two patients Plerixafor was administrated before the second leukapheresis after mobilization with chemotherapy and G-CSF due to very low collected cells count after first leukapheresis.
Three patients underwent one apheresis, three patients two and two patients three aphereses. Median collected CD34+ cells from one apheresis was $3,25x10^6$/kg (0, 31–11, 93). Only in one patient the collected CD34+ cells number after 2 applications of Plerixafor was not sufficient for transplantation (≤2 $x10^6$/kg). Seven of the patients were transplanted successfully after a different long period from collection.
Based on this report and the reviewed literature, we consider G-CSF and Plerixafor to be a suitable combination for PBSC mobilization in patients with different malignant diseases and failures in previous conventional mobilization attempts.

**P722**

**Use of pegylated G-CSF for stem cell mobilisation and harvesting in myeloma patients**

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Myeloma patients undergoing autologous stem cell transplant are traditionally harvested using high dose G-CSF (filgrastim) for stem cell mobilisation. Pegylated G-CSF (Peglasta) is G-CSF bound covalently to a 20kDa PEG molecule which increases the serum half-life of G-CSF due to decreased renal elimination. We present a non-randomised, prospective study of a single-centre experience in 37 myeloma patients who underwent stem cell mobilisation, of which 7 (19%) patients had Peglasta, over the time period from Dec 2006 to Jul 2011. All the myeloma patients were treated with bortezomib-based regimen for 4-6 cycles before stem cell harvesting. After induction therapy with vinorelbine 25 $mg/m^2$ Day 1 and cyclophosphamide 1500 $mg/m^2$ Day 2, each patient was given a single dose of Peglasta 6mg at Day 4 or daily doses of filgrastim at 10mcg/kg/day from Day 4 onwards till stem cell harvesting at Day 8 (at least 5 doses in total). Median patient age was 54 (range 40–67); 12 patients were female. Both Peglasta and filgrastim patients had their stem cell harvesting done at a similar median time of 8 days (7–9) following induction therapy,. During stem cell harvesting, 15 litres (approximately 3 blood volumes) are processed using COBE Spectra® cell separator. Our local institutional minimum CD34+ dose for subsequent double autologous transplant was $6x10^6$/kg. The success rate in achieving the minimum CD34+ dose in the Peglasta group was 42% (3/7) at first day of harvesting and 100% (4/4) at 2nd day of harvesting. In the filgrastim group, the success rate in achieving the minimum CD34+ dose was 33% (10/30) at first day of harvesting, and 90% (18/20) at 2nd day of harvesting. In the Peglasta group, the median CD34+ dose ultimately achieved was 9.94 x $10^6$/kg (6.16–19.87), after a median of 2 aphereses per patient (range 1-3). Compared with the filgrastim group, median CD34+ dose ultimately achieved was 12.8 x $10^6$/kg (5.44–32.83), after a median of 2 aphereses per patient (range 1-3). This study demonstrates that a single dose of Peglasta is comparable to that of filgrastim in the time for stem cell harvesting post induction and success rate in achieving the minimum CD34+ dose. The advantages of using Peglasta are drug savings of approximately US$420 to each individual patient as well as a reduction of 4 days of subcutanous injection. Future studies evaluating the optimal dose, timing of Peglasta as well as long-term outcome in larger cohorts of patients would be required.

**P723**

**Pegfilgrastim is superior compared to filgrastim after high-dose chemotherapy and autologous transplant in patients treated for lymphoma and multiple myeloma**

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Introduction: To reduce the duration of neutropenia after high-dose chemotherapy and autologous peripheral blood stem cell transplantation (APBSCT), granulocyte-colony stimulating factor (G-CSF) is commonly administered. Pegylated filgrastim (pegG-CSF) has different pharmacokinetics than filgrastim (G-CSF) and, therefore, the neutrophil recovery could be faster. Shorter duration of neutropenia, lower risk of severe infection complications, shorter hospitalizations, and lower costs could be the results. The aim of our study was to evaluate the impact of pegG-CSF compared to G-CSF in patients with lymphomas, multiple myeloma, and other gammapathies.
Patients and Methods: We compared 50 consecutive patients treated in our institution from 6/2010 to 7/2011, who received peg-CSF day +1 after high-dose chemotherapy (Mel200, Mel 140, BEAM) for lymphomas, multiple myeloma, and other gammapathies with 51 patients previously treated patients, who received G-CSF from day+7 (80%) or earlier until neutrophil engraftment. Thirteen patients were first transplanted with G-CSF and later with pegG-CSF. Both groups were comparable with no significant difference in age, sex, weight, diagnosis, high-dose chemotherapy, CD34+ cell and CFU-GM doses.
Results: Duration of neutropenia (GRN<0.5) was significantly ($p<0,01$) shorter in pegG-CSF group (2-9, median 5 days) compared to G-CSF group (3-15, median 6 days). Similar results were observed for time to GRN>0.5 (9 *vs.* 11 days, $p<0,001$), GRN>1 ($p<0,001$), and WBC>1 ($p<0,001$). Number of days in hospital after transplant was also significantly lower in pegG-CSF group (9-22, median 13) compared to G-CSF group (11-30, median 14, $p<0,005$). There were no differences in duration of

thrombocytopenia and time to platelet engraftment (>20 and >50) as well as in numbers of days with fever, with iv. antibiotics, and with parenteral nutrition.
Conclusion: Based on our experience we conclude that pegfilgrastim given in a single dose on day +1 after autologous transplant has a superior effect on leukocyte and granulocyte engraftment compared to daily dose of filgrastim as well as on the length of hospitalization after transplantation in patients with lymphomas and gammapathies. The same results were seen in subgroups of patients transplanted first with G-CSF support and later transplanted with pegG-CSF support.

**P724**
**Mobilization of peripheral blood stem cells for allogeneic transplantation by the use of biosimilar Ratiograstim® in healthy donors**
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Introduction: We investigated on two cohorts the efficacy and safety of peripheral blood stem cell mobilization by either the biosimilar or the reference G-CSF.
Material and Methods: We analyzed two cohorts, one cohort of 11 patients and donors receiving the biosimilar Ratiograstim® versus another cohort of 11 patients and donors receiving reference G-CSF for the parameters described below.
Results: In HD receiving subcutan. Ratiograstim®, WBC counts in the peripheral blood ranged from 29.9 G/L to 64.6 G/L (mean 48.9 G/L) and the CD34+ cells count from 19.3/µl to 114.6µl (mean 67.6/µl). In a mean of 1.45 apheresis procedures 172.5x$10^6$ up to 494x$10^6$ CD34+ cells (mean 354.7x$10^6$) were collected. This resulted in CD34+ cell numbers of 2.28x$10^6$ up to 1x$10^7$ (mean 5.17x$10^6$) per kg body weight of the patients. Grafts contained 0.77 up to 2.55 x$10^{10}$ (mean 1.45 x$10^{10}$) CD3+ T cells and 3.71 up to 9.90 x$10^{10}$ (mean 6.98 x$10^{10}$) nucleated cells (NC). In HD receiving subcutan. the reference G-CSF, WBC counts ranged from 27.1 G/L up to 62.5 G/L (mean 43.3 G/L) and the CD34+ cell counts from 13.55/µl up to 122.4/µl (mean 57.3/µl). A mean of 1.27 apheresis procedures were necessary to collect 181.74x$10^6$ up to 598.00x$10^6$ CD34+ cells (mean 370.49x$10^6$) absolutely. This translates into 2.33x$10^6$ up to 7.9x$10^6$ (mean 4.95x$10^6$) CD34+ cells per kg body weight of the patients. Grafts contained 1.01 up to 2.31 x$10^{10}$ (mean 1.55 x$10^{10}$) CD3+ T cells and 3.61 up to 9.55 x$10^{10}$ (mean 5.57 x$10^{10}$) NC. In the Ratiograstim® cohort, patients regenerated WBC >0.5 and >1 G/L within 13 and 14 days, neutrophils >0.5 G/L within 15 days and platelets >50 G/L within 17 days. In the reference G-CSF group, patients regenerated WBC >0.5 and >1 G/L within 14 and 16 days, neutrophils >0.5 G/L within 17 days and pletelets >50 G/L within 15 days. No differences in side effects were observed in both cohorts.
Summary: Efficacy and safety of the biosimilar Ratiograstim®, when compared to the reference G-CSF, was comparable in our cohort of patients in the context of allogeneic stem cell transplantation.

**P725**
**Biosimilar G-CSF (filgrastim) is also efficient for peripheral blood stem cell mobilisation and no cryopreserved autologous transplantation**
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Purpose: The purpose of this study was to compare the mobilization effects of Zarzio® (filgrastim) a biosimilar Granulocyte colony-stimulating factor (G-CSF), versus Neupogen® (filgastrim) for no cryopreserved autologous peripheral blood stem cell (PBSC) collection.
Methods: A total of 20 patients with a hematological malignancy were included. 10 prospectively received biosimilar G-CSF (Zarzio® Sandoz Biopharmaceuticals, Paris, France), compared with a retrospective control cohort ,who had been received between 2009-2011 with G-CSF (Neupogen®, Amgen, Paris, France) following by a dose of chemotherapy and no cryopreserved stem cells.
Results: Median age was 33.5 years (17-64) and 29 years (22-61) in Zarzio® and Neupogen® groups respectively. The two groups were similar in concerning the disease (6 HDK and 4 MM), sex (4 females and 6 males), disease status at graft (8 CR and 02 PR), and previous lines of chemotherapy (at single line in 8 patients and two lines in 2). The first PBSC leukapheresis (Spectra®optia, Caridian BCT®, Lakewood, CO, USA) done on the 5th day. Failure of mobilization was defined by a number of CD34+/kg <2 X$10^6$.
There were no significant differences in outcomes, between groups receiving Zarzio® or Neupogen® when we looked at the number of white blood cell (WBC), after the beginning of mobilization with a median of 24.5 x $10^9$ (range 10.3-56.9) and 25.1 x $10^9$ (range 16.7-63.4) at day 1 and 35.5 x$10^9$/L (26.6-65.4) and 37.5 x$10^9$/L (20.9-67.7) at day 5, median number of leukapheresis necessary to harvest a minimal count of 2x$10^6$ CD34+/kg, 1 (range 1-2) and 1(range 1-3), median of CD34+/kg 4.09x$10^6$ CD34+/kg (range 0.25–4.84) versus 2.52 x$10^6$ CD34+/kg (range 1.22-10.3) (p=0, 7, CI=95%), Bone pain and headache were reported for 3 and 2 patients, and 3 and 1 patients In Zarzio® and Neupogen® groups respectively. In both groups failure of mobilization was at two patients.
In summary, our study shows that Zarzio® is efficient as Neupogen® for the mobilization of CD34 (+) cells, for no cryopreserved autologous PBSC, and it offers an advantage of cost, especially in countries in the process of development.

**P726**
**Mobilisation of heart failure patients using biosimilar granulocyte colony stimulating factor (TevaGrastim) for autologous CD34+ cells preparation and application into the heart**
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Introduction: Granulocyte colony stimulating factor (G-CSF) filgrastim is standard of care in hematopoietic stem cell (HSC) mobilization either as monotherapy or in combination after chemotherapy. Biosimilars such as TevaGrastim were extensively studied in the setting of neutropenia in cancer patients. They were also licensed for hematopoietic stem cell mobilization for which clinical experience is limited.
Methods: We performed autologous HSC transplant intracoronary or intramyocardialy for advanced heart failure in eligible patients on waiting list for the heart transplant. Biosimilar TevaGrastim was used for bone marrow stem cell mobilization according to standard protocol 10 µg/kg body weight daily for five days. On day five CD34+ cell level above 15x$10^6$/l was considered adequate for the start of apheresis. Peripheral HSC collection was performed using Amicus cell separator.
Results: Twenty four patients (men=20) with dilative (n=17) and ischemic (n=7) cardiomiopathy were mobilized with TevaGrastim. Their median age was 59 (24-68) years. TevaGrastim was administered for five (4-6) days. On day 5 the median white blood cell count, CD34+ cell count and their percentage were 46.8 (25.0-70.1) x$10^9$/l, 28.49 (7.5-167.72) x$10^6$/l and 0.11% (0.02-0.37), respectively. Three patients were poor mobilizers not reaching 15x$10^6$/l CD34+ cells level. No serious adverse event was observed with only mild muscle and bone pain (n=4).
Conclusion: Use of biosimilar G-CSF TevaGrastim in HSC mobilization is feasible with success rates similar to filgrastim

according to our experience and historic reports by others with comparable side effects. Further studies in other patient groups and long term follow up are necessary.

**P727**
**Use of a biosimilar G-CSF in allogeneic stem cell mobilisation**
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Objectives: Biosimilar granulocyte colony-stimulating factor (G-CSF) has been approved on the basis of comparable quality, safety and efficacy as the originator product. Approval of biosimilar G-CSF is in the same indications as the originator, including peripheral blood stem cell (PBSC) mobilisation, for which it is being used throughout our large hospital group in Paris, France. Concerns have been raised by professional bodies over use of biosimilar G-CSF in allogeneic transplants. Here we report use of biosimilar G-CSF in healthy donors for allogeneic transplantation.
Methods: Healthy related donors received biosimilar G-CSF (Zarzio®, Sandoz Biopharmaceuticals) 10 µg/kg/day for PBSC mobilisation. G-CSF was administered on days 1-4 with the first leukapheresis performed on day 5. If the required number of CD34+ cells per recipient body weight (4 x 106 CD34+ cells/kg) was not collected, G-CSF administration was continued and a second PBSC collection was performed on day 6. Further G-CSF administration and a third leukapheresis was done on day 7 if required.
Results: A total of 21 healthy donors (13 male, 8 female; mean ± SD age 57.7 ± 11.3 years, range 21-73) received biosimilar G-CSF for PBSC mobilisation. Median donor body weight was 80 kg (range 54-120 kg). Median CD34+ cell count on day 5 was 72/ µll (range 16-145). A single leukapheresis after 4 injections of biosimilar G-CSF was sufficient to collect the CD34+ cell dose required in 11 donors with peripheral blood (PB) CD34+ counts ranging from 69-145 µl. Nine donors underwent a second leukapheresis after 5 days of biosimilar G-CSF, one of whom (a 73-year old female) underwent a third leukaphereses on day 7 (PB CD34+ at first apheresis 23 µl). Median number of CD34+ cells per recipient bodyweight was 6.0 x $10^6$ cells/kg (range 2.6-9.2). Eight donors reported bone pain (4 mild, 1 moderate severity) which was effectively treated with paracetamol. No other adverse events were reported. Blood measurements were normal in all donors when assessed one week after the end of mobilisation. These findings are consistent with our previous use of originator G-CSF (Neupogen®) in allogeneic stem cell mobilisation.
Conclusion: These preliminary findings suggest biosimilar G-CSF is effective and well-tolerated in allogeneic stem cell mobilisation. Further studies are required to assess longer-term safety outcomes.

**P728**
**The effect of cyclophosphamide with bortezomib as the mobilisation regimen on the graft quality and response of disease in multiple myeloma**
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Bortezomib, a proteasome inhibitor has been known to synergize with alkylating agents on myeloma cells. Therefore, we aimed to investigate the adding of bortezomib to high dose cyclophosphamide (Cy) as mobilization regimen in the patients with multiple myeloma (MM).
Patients and Methods: Between April 2010-October 2011, total 10 patients (6 F; 4 M) with MM were given Bortezomib plus Cy as mobilization regimen in our center. Median age was 58.5 years (range: 37-70 ys). Mobilization scheme: Bortezomib at the dose of 1.3 mg/sqm i.v. route was used at the 1st day 1 and 4th day. Cy at the dose of 4g/sqm i.v route was given at the first day following bortezomib. G-CSF, the dose of 10 ug/kg/day, was initiated from day 5 until the completion of stem cell collection. We evaluated to obtain at least 4x$10^6$/kg BW CD34+ cell especially in the first collection. In addition, we analyzed the effect of mobilization regimen on the response of disease prior high dose therapy (HDT) rescued by autologous stem cell transplantation (auto-SCT) and hematopoietic recovery after the transplantation.

**[P728]** **Table 1:** The data of collection and engraftment kinetics

| Pts | The number of apheresis | The number of apheresis for the target | Total CD34+ cells/kg | Infused CD34+ cells/kg | The disease response | | Hematopoietic recovery after the transp. | |
|---|---|---|---|---|---|---|---|---|
| | | | | | At mob. | At the transp. | Neutrophil (>0.5x$10^9$/L) | Platelet (>20x$10^9$/L) |
| 1 | 2 | 1 | 7.4 | 4.02 | PR | PR | 13 | 15 |
| 2 | 2 | 2 | 6.4 | 3.05 | PR | CR | 11 | 17 |
| 3 | 1 | 1 | 21 | 7 | VGPR | CR | 12 | 12 |
| 4 | 1 | 1 | 19.2 | 9.6 | PR | PR | 18 | 24 |
| 5* | 1 | 1 | 14.6 | 7.3 | PR | PR | 12 | 13 |
| 6 | 2 | 2 | 4 | 4.0 | VGPR | CR | 12 | 16 |
| 7* | 3 | 1 | 5.9 | 5.9 | CR | CR | 12 | 11 |
| 8 | 1 | 1 | 6.9 | 5.8 | PR | nCR | 12 | 12 |
| 9 | 1 | 1 | 37.0 | 16.6 | VGPR | CR | 12 | Not drop |
| 10 | 1 | 1 | 13.7 | 7.3 | PR | CR | Not transplanted yet | |

*The mobilization regimen was used in 5th and 7th patients for relapse after HDT.
Abbreviations: CR: Complete response; PR: Partial response; nCR: Near Complete response; VGPR: Very Good Partial Response.

Results: The scoring of the patients as IPI at the diagnosis was between I-III. Two out of the patients received the mobilization regimen for the disease relapse following first autologous transplantation. Adequate quantities of CD34+ cells were collected with one and three apheresis procedures (median: 1) at median 12 days (range: 11-22 days) after the beginning of the mobilization regimen in all the patients (Table 1). The quantity of targeted CD34+ cells (>4x10$^9$/kg) with first collection was reached in 8 patients (80%). Febrile neutropenic episode was seen in 5 patients after mobilization regimen. Transfusion supports, packed red blood cells or platelets were required in only 5 patients. Complete responses from partial or very good partial remission were obtained in 6 patients (60%) after mobilization regimen. All of the patients but one underwent the HDT supported by auto-HSCs. Hematopoetic recoveries for neutrophil or platelet were observed in median 12 days and 12.5 days, respectively.
In conclusion, the adding of bortezomib to high dose Cy did not decrease the stem cell content and led to adequate cytoreduction for the disease. Further no negative impact on hematopoetic recovery after the transplantation was seen. The effect of Bortezomib on stem cell content, hematopoietic recovery and the disease response should be evaluated in a prospective randomized study including Cy alone versus the combination with Bortezomib.

**P729**
**Satisfactory mobilitation with filgrastim (granulocyte colony-stimulating factor) as a single agent in autologous haematopoietic progenitor transplantation. A single-centre experience**
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Introduction: The number of CD 34 + cells collected is essential for proper hematopoietic graft in autologous hematopoietic stem cell transplantation (auto-HSCT). To mobilizate hematopoietic progenitors (HP) changes of mechanisms of adhesion and microenvironment are needed. The granulocyte colony-stimulating factor (G-CSF) with chemoterapy have been used as mobilizers to obtain HP. G-CSF has proven effective as a single mobilizing agent altering these adhesion mechanisms.
Methods: We reviewed data of mobilization with G-CSF as a single agent in transplant patients (auto-HSCT) in our center over a period of 13 years (1997-2010). All HP apheresis were performed with the cell separator CS-3000plus Fenwall (Baxter). We reviewed a) variables prior to mobilization: diagnosis, sex, age, prior chemotherapy, b) variables related to mobilization: mobilization schemes with G-CSF, number of mobilizations, apheresis number and CD34+/kg number obtained (measured by flow cytometry), and c) post mobilization and autologous transplantation variables: median graft days for neutrophils (> 0.5 x 10e9 / L) and platelets (> 20 x 10e9 / L on two consecutive days).
Results: 125 patients were mobilized, 84 men and 61 women, who subsequently underwent auto-HSCT. The median age was 52 (15-69) years. The diagnoses were: a) Multiple Myeloma (n=64), b) non-Hodgkin lymphoma (n=36), c) Hodgkin lymphoma (n=20), d) acute leukemias (n=17), e) plasma cell leukemias (n=4), f) Solid tumors (breast and germ) (n=4). The median number of previous treatment lines was 2 in all conditions except in Multiple Myeloma (MM), that was 1 (in 89% of MM). The mobilizing scheme used was G-CSF at doses of 10 µg/kg/12h, collecting on the fifth day. The type of access used was Hickman catheter (73%) and Quinton-Mahurca (27%). The mean volemia processed was 2, needing a single apheresis in 115 patients. 14 requires 2 mobilizations. The average CD34 +/kg collected was 2.7 (1-9) x 10$^6$/kg of actual weight. Average days to neutrophil and platelet recovery was 12 and 17, respectively, without registering any graft failure.
Conclusions: Our experience shows that mobilization with G-CSF is a simple and effective method for obtaining an adequate number of CD34 for leukocyte and platelet graft in auto-HSCT, independently of diagnosis and treatment used.

**P730**
**Successful stem cell mobilisation and autologous stem cell transplantation after bendamustine pretreatment in patients with multiple myeloma**
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Bendamustine is a bifunctional alkylating agent with promising activity in Non-Hodgkin-Lymphoma and Multiple Myeloma (MM). A prospective, randomized, phase III trial demonstrated that bendamustine in combination with prednisone (BP) was superior to the standard combination of melphalan/prednisone (MP) with respect to complete response rate, mean number of cycles to maximum response, time to treatment failure and quality of life in patients with newly diagnosed MM (Poenisch *et al*, Res Clin Oncol 132: 205-212;2006). Unfortunately, the previous development of this agent did not provide sufficient information on stem cell toxicity and mobilization of stem cells for autologous stem cell transplantation (SCT) after Bendamustine therapy. A retrospective analysis of peripheral blood stem cell mobilization and autologous SCT was performed in 63 patients with multiple myeloma who had received Bendamustine pretreatment at the university Hospitals Leipzig and Heidelberg over a period of sixteen years. Patients had a median age of 59 (range, 31-72) years. The cumulative dosis of Bendamustine per patient was 960 (range 120–2400) mg/qm and was administered during a median of three (range 1-10) cycles. The mobilization regimen consisted of Cyclophosphamide 4g/qm (n=41) or 7g/qm (n=4) and G-CSF (2x5ug/kg). Alternative regimens such as CAD, CED, TCED and others were also used in the remaining patients. Apheresis was started as soon as peripheral blood CD34+ counts exceeded 10x10$^6$/l with a harvest target of 4x106 CD34+/kg using 4 times the blood volume. The minimal accepted target was 2x10$^6$ CD34+/kg. Stem cell mobilization and harvest was successful in 60 of the 63 patients (95%). In 19 of 60 patients (32%) a single apharesis was sufficient to reach the target. The median number of aphareses was two (range 1-7) and the median CD34+ cell-count/kg was 5.9 (range 1.7-20.4) x10$^6$. Information on autologous SCT is available from all 60 patients with successful harvest. Engraftment was successful in 59 of 60 patients. The median time to leucocytes count >l×10$^9$/l was reached after 12 days and the time to untransfused platelet count of >50×10$^9$/l was 14 days. 54 patients (90%) responded after the autologous SCT with 6 CR, 4 nCR, 12 VGPR, and 32 PR. The event free survival at 36 months was 31% and overall survival was 68%. In conclusion, the stem cell mobilization and autologous SCT is feasible in multiple myeloma patients who have received Bendamustine pretreatment.

**P731**
**A retrospective comparison of PBSCC yield following CTD or (C)VAD'**
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Standard treatment of Multiple Myeloma has changed with combination regimes of Vincristine, Adriamycin and Dexamethasone (VAD) being superseded by newer chemo-therapeutic approaches or novel agents. Consolidation of first line

treatment with autologous stem cell transplant (SCT) is still considered treatment of choice. However, the impact of novel agents upon peripheral blood stem cell collection (PBSCC) has not yet been explored. This small centre retrospective study of PBSCC yields in patients who have only received one line of treatment, Cyclophosphamide, Thalidomide and Dexamethosone (CTD), VAD or CVAD (with the inclusion of Cyclophosphamide). Local anecdotal evidence suggested that collection and yield were not as successful in patients who received CTD as compared with VAD/CVAD. Clinical discussion appeared to indicate that the combination of Cyclophosphamide and Thalidomide may inhibit stem cell mobilisation. Data on CD34 yield obtained at apheresis were collected for patients attending NHSBT for PBSCC from 2006 to 2011. Sixty five patients were identified, representing 87 PBSCC episodes. These patients were subdivided by first line therapy received. Patients who had received multiple chemotherapy regimes prior to collection were excluded from the analysis. The desired therapeutic yield for each patient was a minimum of CD34 2.0. Twenty six patients were identified as having received CTD only prior to PBSCC. This accounted for 36 PBSCC episodes, averaging 1.38 collections per person to achieve minimum therapeutic dose. Four patients failed to achieve minimum collection and did not proceed to SCT. The average yield per patient for the CTD group was 3.03; 2.19 per PBSCC episode. The yield obtained in a single PBSCC episode (duration 1-3 days) ranged from 0.82 to 6.97, excluding those who failed to collect a measurable amount of stem cells. The comparison group was patients who received either VAD or CVAD at first line. This cohort is 11 patients. They represent 13 PBSCC episodes with a mean yield per collection of 3.26 and per person of 3.86. These figures are notably higher than that the mean yields obtained in the CTD group. Additionally, the VAD or CVAD group had a lower incidence of failed collection and a mean of 1.18 collections per patient. Analysis found that the CTD cohort were twice as likely to require second mobilisation to achieve minimum therapeutic CD34 count.

**P732**

**Kinetics of CD34+ cells during mobilisation using CTX plus G-CSF allows for an early identification of "not mobilizing" patients and can be used as criteria for early "on demand" use of plerixafor**

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Identification of factors able to predict mobilization failure could lead to use of Plerixafor already during first mobilization, avoiding repetition of a second mobilization course. Clinical features already known at baseline, before start of mobilization, fail, however, to predict "failure of mobilization" in a sustantial number of patients. No data are available on predictive value of other parameters generated early during first mobilization attempt. To address this issue, we studied the value of CD34 counts in PB measured early, at day +10, +11, +12 and +13, in predicting the mobilization outcome in the ensuing days. We have retrospectively collected data on patients at first or second attempt of mobilization, affected by Multiple Myeloma or by Lymphoma that received as mobilizing treatment cyclophosphamide 4 gr/mq and G-CSF. Variability on kinetics of CD34 upsurge was studied in two hundred and thirty-three patients (Figure 1). To measure diagnostic value of PB CD34 value in respect of "failure of mobilization", we considered a failed mobilization as "disease" and value of CD34+ cells, at a specific day, in PB, as a "diagnostic test". We measured sensitivity (n° of patients who had a positive test and a failed mobilization/all patients who failed mobilization); false positive rate (n° of patients who had test positive and a successful mobilization/ all patients who had a successful mobilization); specificity (negative test and successful mobilization/all patients who had a successful mobilization) and positive predictive value (n° of patients who had a positive test and a failed mobilization/all patients with positive test). Different threshold of CD34 cells (CD34 in PB= 6 cells/mmc and 10 cells/mmc) at different time point (day 10th-11th -12th -13th) lead to results reported in table 1. A count of CD34+ cells below 10/mmc at day 13th had a high sensitivity and high specificity in predicting a failure of mobilization. However, good predictive result was obtained using a lower threshold (CD34+: 6 cells/mmc) at a earlier time. Thus, to start "early on demand" Plerixafor for PBSC mobilization, it can be proposed to use a level of CD34 cells below 6/mmc at day 12th or a CD34 cell level below 10 cells/mmc at day 13th. The criteria we proposed to start early "on demand Plerixafor" have high positive predictive values and are, therefore, able to avoid an inappropriate use of this expensive agent.

**P733**

**Predictive factors for a failed CD34 mobilisation or a poor CD34 harvest in multiple myeloma patients treated by new agents and mobilised using CTX 4 gr/mq + G-CSF**

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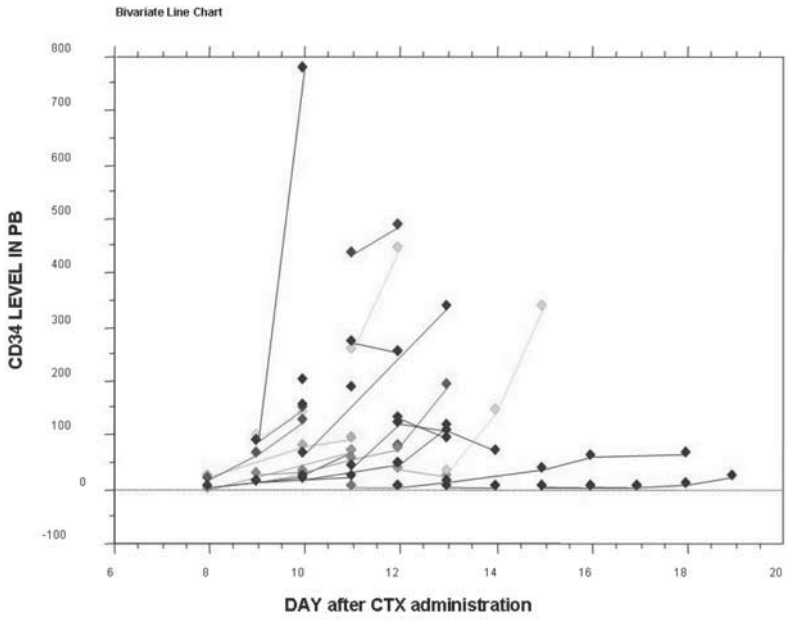
The place of Plerixafor in Myeloma (MM) patients is still a matter of debate. It is critical to know to which extent CD34+ mobilization failure or a poor CD34 harvest can be predicted in Myeloma (MM) patients using data available at start of mobilization. We evaluated in a retrospective study, factors already known at start of mobilization (baseline) for their predictive value in respect of failure of PBSC mobilization. Selection criteria was: diagnosis of MM, age below 70 years, first attempt of PBSC mobilization done using CTX 4 gr/m$^2$ plus G-CSF, mobilizing attempt done during the years 2006-2011. A total of 171 mobilization were selected. As induction treatment, 98 patients received lenalidomide or thalidomide. Criteria used to start of apheresis was a PB CD34 value over 20 mmc. Logistic regression was utilized to study factors predictive for:
a) a successful CD34+ cells mobilization (defined as a CD34 count in PB>20 cells/mmc).
b) an harvest sufficient for at least one High Dose treatment (defined as CD34+ dose >2x10e6/Kg).
c) an harvest sufficient for two High Dose treatments (defined as CD34+ dose >5x10e6/Kg).
A failure of CD34 cell mobilization in PB was registered in 6% of patients, a failure to collect cells for 1 high dose treatment in 12.5% of all patients, a failure to collect cells for 2 high dose courses was registered in 36%.
A) A successful CD34 mobilization was not predicted by any factors. B) The harvest of a CD34 dose > 2x10e6/Kg was predicted in univariable analysis by male gender (p=0.01), WBC at baseline (p=0.01), number of apheresis (p=0.05, in multivariable logistic regression only number of apheresis remained important (p=0.01). C) A failure to harvest an optimal dose(CD34 dose > 5 x10e6/Kg) was associated, in univariate analysis, with: lower dose of G-CSF (p=0.003), residual infiltration at BM biopsy over 10% (p=0.02), a Platelets count below 140x10e9/L (p=0.003) and being treated in center n.1 and n.2 in respect to center n.3 (p=0.0001); in multivariable analysis the only predictive factor was a Platelets count below 140x10e9/L (p=0-04).
In conclusion, 1) in patients affected by MM, PBSC mobilization and harvest is not influenced by previous treatments with new biologic active drugs such as thalidomide or lenalidomide nor by G-CSF dosage, no detrimental effect of limited vertebral RT

[P732]




| DAY | CD34 level in PB | Sensitivity in prediction of a "failed mobilization" | False positivity in prediction of a "failed mobilization" | Specificity in prediction of a "failed mobilization" | True positive predictive value (PPV) |
|---|---|---|---|---|---|
| D 10 | 6,00 | 50 | 2 | 97 | 57 |
| D 11 | 6,00 | 69 | 1 | 98 | 90 |
| D 12 | 6,00 | 87 | 4 | 95 | 70 |
| D 13 | 6,00 | 75 | 0 | 100 | 100 |
| D 13 | 10,00 | 100 | 0 | 100 | 100 |

is also apparent. 2) Factors able to predict for sufficient mobilization are not easily identifiable at baseline.

**P734**

**Very high efficacy of first- and second-line stem cell mobilisation using intermediate-dose cytosine arabinoside with G-CSF**

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High-dose chemotherapy with autologous peripheral blood stem cell transplantation (autoPBSCT) is a standard of care in patients with multiple myeloma (MM) as well as poor risk Hodgkin (HL) and non-Hodgkin lymphoma (NHL). However, a significant proportion of patients fail to mobilize stem cells after G-CSF alone or cyclophosphamide (CTX) + G-CSF, being most commonly used protocols.

In this study we evaluated efficacy of mobilization based on cytosine arabinoside (Ara-C) administered at a dose 2x400 mg/m$^2$ on days 1, 2 (3) (total dose 1600-2400 mg/m$^2$) + G-CSF (filgrastim) 5-10 ug/kg starting from day 5.

In the first phase the protocol was used as a salvage regimen in 13 patients (MM, 5; HL, 2; NHL, 3) who failed to mobilize after CTX + G-CSF (n=10) or combined chemotherapy regimens (n=3). All patients mobilized >2x10e6 CD34+ cells/kg (median 10.3, range 2.5-25.9), which was achieved with one (n=8) or two (n=5) leukaphereses.

In the second phase we evaluated AraC+G-CSF as a first-line mobilization in a prospective cohort of 51 patients with MM (n=32), HL (n=3) and NHL (n=26). Median age was 56 (26-69) years. Patients had previously been treated with a median of 7 (4-36) cycles of chemotherapy, which in case of MM included thalidomide. 25 patients (49%) had been administered at least 2 lines of chemotherapy and 18 (35%) had received radiotherapy. All but one patient (98%) reached >15 CD34+ cells/uL in peripheral blood, which was required to start leukapheresis. Median level was 136 (5-780) CD34+ cells/uL. Leukaphereses started on day +14 (11-17). 50 (98%) patients collected ≥2x10e6 CD34+ cells/kg with the median 15.6 (2-65.1), which was achieved with a single leukapheresis in 45 (90%) patients. 46 (90%) patients collected ≥4x10e6 CD34+ cells/kg required for tandem autoPBSCT. Results were compared with the historical control of 67 unselected patients with similar clinical characteristics, in whom mobilization was based on CTX (total 1.5-4 g/m2) +G-CSF. AraC+G-CSF was found significantly more effective (see Table).

[P734]

| | AraC +G-CSF (N=51) | CTX+G-CSF (N=67) | p |
|---|---|---|---|
| Max. CD34+ cells/uL in peripheral blood | 136 (5-780) | 29 (0-240) | <0.0001 |
| >15 CD34+ cells/ul in peripheral blood | 50 (98%) | 48 (74%) | 0.0002 |
| CD34+ cells/kg collected | 15.6 (2-65.1) | 5.2 (0.4-14.3) | <0.0001 |
| >2 x10e6 CD34+ cell/kg collected | 50 (98%) | 43 (64%) | <0.0001 |
| >4x10e6 CD34+ cells/kg collected | 46 (90%) | 34 (51%) | <0.0001 |
| Single leukapheresis sufficient | 45/50 (90%) | 17/55 (31%) | <0.0001 |

We conclude that mobilization based on intermediate doses of AraC followed by G-CSF is highly effective allowing adequate CD34+ harvest in almost all patients with lymphoproliferative diseases, including heavily pre-treated individuals and those failing previous mobilization. The level of mobilized CD34+ cells is four times higher compared to protocol based on CTX+G-CSF.

**P735**

**CD34±selected stem cell boost without further conditioning for poor graft function after allogeneic stem cell transplantation in patients with haematological malignancies**

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Poor graft function (PGF) is defined as an inability to achieve adequate blood counts in at least two lines after allogeneic HSCT in the presence of complete donor hematopoietic cell chimerism and absence of viral reactivation, signs of GvHD or drug-related myelosupression. It could be primary, developing in the first 28 days post-transplant, or secondary in case engraftment had already been achieved. The incidence of PGF after allogeneic HSCT varies from 5% to 27% in published reports. In this 2 center retrospective study we investigated the use of CD34±selected stem cell boost (SCB) without prior conditioning in 32 PGF patients (pts, male, n=22, female, n=10; median age of 54 years (20-69)). The median interval between HSCT and SCB was 5 months (2–228). The median amounts of CD34+ and CD3+ cells were $3.4x10^6$/kg b.w. (0.96–8.30) and $9x10^3$/kg b.w. (2–70). Hematological improvement (HI; neutrophils $>1.5x10^9$/L, platelets $>30x10^9$/L and hemoglobin >8.5 g/dL) was observed in 26/32 pts (81%). Almost 40% of pts showed signs of HI already at day +14 after boost. The recipients of related grafts responded earlier (day +20 *vs.* day +30, p=0.039) and more frequently (100% *vs.* 71%, p=0.07) than recipients of unrelated grafts. Pts experienced hematological response (neutrophils $>2.5x10^9$/L, platelets $>100x10^9$/L and hemoglobin >10 g/dL) were younger as non-responding pts (39 *vs.* 54 years, p=0.041) and received grafts from younger donors (35 *vs.* 43 years, p=0.036). The CD34+ and CD3+ cell doses had no impact on the HI/response. The incidence of acute and chronic GvHD was 17% and 26%, respectively. Pts with acute, but not chronic GvHD received a significantly higher median CD3+ cell dose (32 *vs.* $8x10^3$/kg b.w., p=0.02). At a median follow up of 30 months (4 – 107) a number of 16 pts (of those 5 no-responders) had died. The causes of death were: sepsis, n=8; aGvHD, n=3; cGvHD with concomitant infection, n=1; relapse/disease progression, n=3; cerebral hemorrhage, n=1. The 1-year probabilities of OS and PFS were 57% and 56%, respectively. The achievement of HI had a positive impact on the OS (HR 2.7; p=0.08).
In conclusion, the use of CD34+ selected SCB without chemotherapeutic or immunosuppressive conditioning is associated with high and fast hematopoietic improvement. Nevertheless, risk of acute GvHD should be taken into account. Further studies are needed to evaluate a role of immunosupression folowing the CD34±selected boost administration.

**P736**

**Activation of haemostasis after transfusion of cryopreserved haematopoietic stem cells containing DMSO does not affect engraftment**

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Objectives: Investigation of the toxicity of DMSO-containing cryopreserved hematopoietic stem cells (HSC) on hemostasis by determination of platelet function and of coagulation and endothelial cell activation in patients receiving autologous HSC transplants (HSCT).
Patients and Methods: 54 patients (21 female, 33 male, age 55±13 years) received autologous HSCT containing 7,5% DMSO. Underlying diseases were multiple myeloma (n=29), lymphoid neoplasia (n=14), acute leukemia (n=5), others (n=6). Blood samples were taken immediately before, 15 min and 16-18 hours after HSCT. We tested whole-blood platelet aggregation (adenosine-diphosphate [ADP 6.4mcM], collagen [COL 3.2 mcg/ml], thrombin receptor activating peptide [TRAP 32 mcM], and arachidonate [AA 0.5 mM]), fibrinogen (FBG), d-dimers (DD), thrombin-antithrombin complex (TAT), von Willebrand factor antigen (VWF: Ag), and cell-membrane microparticle activity (MPA) measured as thrombin-generation.
Results: Mean MPA (16±9 nM, 27±18 nM, 14±8 nM, p<0.001) and TAT (4±2, 17±13, 4±4 mcg/L, p<0.001) increased significantly immediately after HSCT, returning to baseline the day after. Minor but significant changes were seen in FBG, DD and VWF:Ag. Only a trend for reduction of platelet aggregation with ADP (449±263, 380±277 AU, p=0.06) was found immediately after HSCT. Ten of 54 (19%) patients presented fever and/or urticaria immediately after transfusion of HSC, accompanied by higher VWF (p<0.01) but not DD, MPA, FBG or TAT changes. Engraftment was seen at 15±7 days, no differences were found in patients with high increase of TAT or MPA. Patients with high increase in TAT or MPA had received significantly higher numbers of total cells and higher transplant volumes.
Discussion: We observed an activation of hemostasis, measured as increase of MPA and TAT immediately after HSCT. This effect is short-lasting and might be attributed to cell-components of the HSC preparation and/or DMSO. Engraftment was

not affected by this phenomenon. Known adverse reactions to transfusion of cryopreserved HSC, such as fever and/or urticaria were not associated with higher DD, MPA or TAT changes.

**P737**

**A paired comparison of autologous blood haematopoietic stem cell collections in 18 patients undergoing aphaeresis, alternatively using the OPTIA and SPECTRA cell separators, reveals a lower neutrophil content in products collected with OPTIA**

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The main criteria to assess quality of autologous blood stem cell products that are obtained as the first step for intensive chemotherapy supported with autologous transplantation is their content in CD34+ progenitors. However, CD34+ cells represent only a minor fraction of collected cells that are diluted in maturing and terminally differentiated cells, especially of the monocytic and neutrophil lineages. We and others have shown that contamination of collected blood cell therapy products with with high numbers of cells of the neutrophil lineage produce deleterious effects at various stages of subsequent processing, as well as predispose to the occurrence of grade III-IV adverse events at the time of infusion.
We compared the neutrophil contamination of paired aphaeresis products alternatively obtained by using the Terumo BCT SPECTRA (semi-automated program v4.7) and OPTIA cell separators in 18 adult patients (9 with lymphoma, 3 with myeloma, 5 with solid tumors and 1 with AML) candidate for high-dose chemotherapy and autologous transplantation at a single JACIE-accredited program. Ten patients were collected during the G-CSF supported recovery phase of cytopenia induced by myelo-suppressive chemotherapy ("chemotherapy + rhG-CSF"). 8 patients were collected after mobilization with rhG-CSF alone. The average numbers of neutrophils were $32 \times 10^9 \pm 25.5$ and $51.7 \times 10^9 \pm 51.2$ in products collected with the OPTIA and SPECTRA cell separators respectively ($p = 0.09$). When focusing on the subset of 8 patients mobilized with rhG-CSF who displayed more stable blood counts over the two consecutive days when they underwent aphaeresis, a similar difference was observed ($22.8 \times 10^9 \pm 17.8$ and $38.4 \times 10^9 \pm 27.1$; $p=0.08$). The percentages of neutrophils in aphaeresis products were not different with the two cell separators. These preliminary results deserve further confirmation; they however suggest an advantage for the OPTIA cell separator in terms of neutrophil contamination in aphaeresis products.

**P738**

**High expression levels of vla-4 (CD49d) on mobilised peripheral blood stem cells are associated with rapid engraftment in multiple myeloma patients receiving autologous transplant**

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Introduction: Autologous peripheral blood stem cell transplantation (PBSCT) is considered the gold standard for the treatment of newly diagnosed multiple myeloma (MM) patients less than 65 years old. Although CD34+ cell number is the most important factor in predicting neutrophil and platelet engraftment, adhesion receptor molecules on stem cells have been shown to modulate homing and engraftment after transplantation.
Objectives: To define and correlate the pattern of different cell adhesion receptor expression by the CD34+ cells from apheresis products with the time to neutrophil and platelet engraftment in MM patients undergoing autologous PBSCT. Patients and methods: A total of 22 newly diagnosed MM patients (12 male, 10 female; median age 51 years, range 32-70) who received autologous PBSCT were retrospectively evaluated. According to ISS, 10 pts (45.5%) were in stage I, 7 pts (31.8%) in stage II and 5 pts (22.7%) in stage III. Different induction based therapies were used: lenalidomide (5 pts), bortezomib (7 pts) and VAD like (10 pts). Flow cytometry analysis was performed on cryopreserved apheresis samples, using the following three colours antibodies combinations (FITC/PE/PerCP): CD34/CD11a/CD45, CD34/CD44/CD45, CD34/CD49d/CD45, CD34/CD49e/CD45, CD34/CD184/CD45. All CD34+ cell subsets were correlated with the kinetics of early haematopoietic engraftment following auto-PBSCT.
Results: All patients undergoing autologous PBSCT received at least $2.0 \times 10^6$ CD34+ cells/kg. Median CD34+ cell number/kg infused was $4.90 \times 10^6$/kg (range: 2.50-13.80). The median time to achieve early engraftment of neutrophils (ANC>500 µL) and platelets (plt>20000 µL) was 11 days (range 9-14) and 13 days (range 9-30), respectively. Spearman correlations showed that higher expression of CD34+/CD49d+ significantly correlated with more rapid neutrophil and platelet engraftment after transplant ($r=-0.44$, $p=0.03$ for ANC and $r=-0.50$, $p=0.01$ for plts, respectively). In addition, the number of infused CD34+/CD11a+ ($p=0.02$), CD34+/CD49e+ ($p=0.02$) and CD34+/CD184+ ($p=0.03$) better correlated with the time to plt engraftment than that of infused CD34+ cells ($p=0.05$).
Conclusion: Haematopoietic stem cells infused expressing high level of CD34+/CD49d+ best predicted early neutrophil and platelet reconstitution in MM patients after autologous transplant.

**P739**

**Large volume apheresis is efficient independently of the pre-apheresis amount of CD34+ cells**

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Background and Objectives: In the mobilization of hematopoietic progenitor cells from the bone marrow, apheresis has been described to have a mobilizing effect, so that the processing of more than three volume blood volumes increase the yield of peripheral blood progenitor cells (PBPCs).
For this reason large volumes apheresis (LVA) are processed in some centres. The aim of this study is to establish if LVA is equally efficient in patients with low pre-apheresis CD34+ cells than in those with higher counts.
Material and Methods: LVA performed in the past ten years in our hospital are reported. Four times the calculated blood volume have been processed in each apheresis. Mononuclear cells (MNC), CD34+ cells and clonogenic CFU-GM and BFU-E measures were performed: in peripheral blood pre-apheresis, in the product obtained after the first two volumes (1st bag) and after the second two volumes (2nd bag). Recovery of these variables in each one of the two bags was calculated as percentage of the pre-apheresis values; and the results of the two bags were compared.
Results: A total of 517 apheresis, performed to 352 patients, were included; the median of the pre-apheresis CD34+ cells was 8.8/microL. Results are shown in the table. Curiously, the recovery of CD34+ cells in both bags was significantly higher (111 % and 146%) in the group lower than in the higher pre-apheresis (86% and 99%) CD34+ cells, $p<0.001$. The cell cultures gave variable results; whilst CFU-GM recovery was higher

[P739]

Comparison of the recoveries between CD34$^+$ cells pre-apheresis ≥ and < than the median.

| | | CD34 ≥8.8/μL (n=258) | CD34 < 8.8/μL (n=249) | p |
|---|---|---|---|---|
| **CMN** Recovery | 1st bag | 304.52±153.33 | 193.83±36.54 | ns |
| | 2nd bag | 327.06±58.61 | 187.54±18.14 | 0.028 |
| **CD34** Recovery | 1st bag | 86.72±5.11 | 111.67±6.57 | < 0.001 |
| | 2nd bag | 99.23±3.44 | 146.28±8.30 | < 0.001 |
| **CFU-GM** Recovery | 1st bag | 858.97±262.88 | 269.64±32.64 | 0.02 |
| | 2nd bag | 457.07±96.81 | 398.28±66.30 | ns. |
| **BFU-E** Recovery | 1st bag | 197.86±36.10 | 281.36±46.17 | ns |
| | 2nd bag | 203.51±27.38 | 459.77±102.30 | 0.01 |

Values are expressed as mean±standard error of the mean. ns= no signiticant

in the group with more basal CD34+ cells, BFU-E recovery was lower in the same group.
Conclusions: LVA is equally effective in all patients, independently of the basal amount of CD34+ cells, allowing the recovery of significant number of PBPCs in poor mobilizer patients (almost 20% of all cases with autologous transplantation indication). Our results agree with the mobilizing role of the apheresis itself.

**P740**
**To freeze or not to freeze haematopoietic stem cells prior to allogeneic transplantation**

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Background: In order to preserve 100% viability of peripheral blood hematopoietic stem cells (PBSC), the standard practice in allogeneic stem cell transplant in most centers is to infuse PBSC collected the same day or the day before transplantation (SCT). To obtain and freeze PBSC prior to SCT would be desirable in order to get a better logistic and to confirm the availability and quality of the product in unrelated SCT prior the exposure of the patient to the conditioning regimen. Unfortunately comparative studies are lacking AIM: In this retrospective study (period study 1997-2011) we report all products consecutively obtained in three centers with different policy (Cryopreservation versus fresh infusion) to analyze the impact of using fresh (FSH) (N:107) or previously frozen (FRZ) PBSC (N 224) on overall outcomes in the setting of alloPBSCT from a matched related donor.
Results: Both groups were paired in global characteristics although patients in the FSH group had a higher median age (47 *vs* 38) and more commonly received reduced intensity conditionings. Total CD34+ cells infused were slightly higher in the FRZ group (5.6 *vs* 5 x 10e6/kg, p=0.04), and engraftment (granulocytes more than 500/ml) were achieved earlier in the FRZ Group (14 *vs* 16 days, respectively; p=0.001), with no significant differences on platelet recovery (13 days for both). Patients receiving FRZ PBSC had a higher incidence of acute graft versus host disease (aGVHD) (63 *vs* 44%, p <0.001) mostly involving skin and liver, and at an earlier onset (13 *vs* 30 days, p <0.001). Grades II-IV aGVHD was more common in FRZ group (45 *vs* 30%, P 0.008). 28/57 (49%) *vs* 3/44 (13%) of cases of cutaneous aGVHD were diagnosed before 14 days in the FRZ group. Response to first line treatment with corticoids was similar in both groups (87 *vs* 80% in FRZ and FSH group respectively). No statistically significant differences were found regarding chronic GVHD (58 *vs* 66%) nor overall survival (44 *vs* 48%), disease free- survival (39 *vs* 33%), non-relapse mortality (26 *vs* 19%) and relapse rates (29 *vs* 32%) in FRZ *vs* FSH group, respectively.
Conclusions: Infusion of previously FRZ stem cells allows obtaining similar overall outcomes as compared to FSH infusion, in terms of engraftment, NRM and survival, allowing to program apheresis to the donor more easily and to have the product available before the patient undergoes conditioning regimen. Possible mechanisms of cryopreservation influencing on the different pattern of aGVHD should be further investigated.

**P741**
**Analysis of blood grafts after various mobilisation methods in myeloma patients**

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Background: Cyclophosphamide plus G-CSF is commonly used to mobilize stem cells in patients with multiple myeloma (MM). Plerixafor may be added to G-CSF in patients who mobilize poorly or can be used in addition to chemomobilization to boost mobilization. Limited data is available on graft content collected after various mobilization methods in myeloma patients.
Patients and Methods: Altogether grafts collected in 21 MM patients were analyzed. We analyzed CD34+ subclasses, lymphocyte subsets and plasmacytoid dendritic cells from cryopreserved grafts collected on the next morning after plerixafor injection in 10 MM patients mobilized with G-CSF with or without preceding cyclophosphamide. As controls we had the first collections from 11 MM patients mobilized without plerixafor; all had received cyclophosphamide plus G-CSF.
Results: The median CD34+ content of the analyzed grafts was 2.1 x $10^6$/kg in the plerixafor group compared to 4.0 x$10^6$/kg in the controls (p = 0.006). The proportion of the most primitive stem cells (CD34+, CD133+, CD38-) from all CD34+ cells in the graft was higher in the plerixafor group (3.5% *vs.* 0.4%, p = 0.001). The amount of CD3+, CD4+, CD8+ and CD19+ lymphocytes as well as NK cells in the graft tended to be higher in the plerixafor group. Proportion of nonviable lymphocytes and the amount of plasmacytoid dendritic cells was comparable between the groups. The detailed data is presented in Table 1.
Altogether 18 patients have been treated with high-dose chemotherapy. No treatment-related mortality was seen. The median time to reach neutrophils >0.5 x $10^9$/L was 11 days in the plerixafor group and 10 days in the control group. Time to reach platelets >20 x $10^9$/L was also comparable (11 d *vs.* 10 d, respectively.).
Conclusions: Plerixafor seems to have an effect on graft cell composition in myeloma patients mobilizing poorly with traditional regimens. A clinical relevance of these findings is unclear and they need to be confirmed in larger studies.

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**Table 1. CD34+ subclasses, lymphocyte subsets and plasmacytoid dendritic cells in grafts collected after plerixafor injection in myeloma patients mobilizing poorly and in patients mobilized without plerixafor.**

| | plerixafor median (range) | no plerixafor median (range) | *p* |
|---|---|---|---|
| CD34+ cell content(x 10^6/kg) | 2.1 (0.3 – 5.0) | 4.0 (1.2 – 8.4) | 0.006 |
| Proportion of CD34+, CD133+, CD38- cells from all CD34+cells (%) | 3.5 (0.2 – 7.1) | 0.4 (0.1 – 2.3) | 0.001 |
| CD34+, CD133+, CD38-cell content (x 10^6/kg) | 0.036 (0.004 – 0.144) | 0.017 (0.001 – 0.084) | 0.159 |
| CD3+ cell content (x 10^6/kg) | 78.0 (11.6 – 333.9) | 37.1 (6.4 – 90.7) | 0.078 |
| CD3+CD4+ cell content (x 10^6/kg) | 47.6 (8.9 – 206.5) | 25.8 (3.6 – 68.9) | 0.057 |
| CD3+CD8+ cell content (x 10^6/kg) | 24.0 (2.7 – 125.1) | 11.6 (2.8 – 23.1) | 0.275 |
| CD19+ cell content (x 10^6/kg) | 2.6 (0.3 – 56.0) | 0.6 (0.1 – 53.7) | 0.062 |
| NK (CD3-CD16/56+) cell content (x 10^6/kg) | 4.5 (1.1 – 27.6) | 2.4 (1.0 – 10.8) | 0.192 |
| Proportion of plasmacytoid dendritic cells (%) | 0.33 (0.02 – 1.18) | 0.41 (0.20 - 0.63) | 0.360 |
| Plasmacytoid dendritic cell content (x 10^6/kg) | 1.3 (0.2 – 4.6) | 0.8 (0.2 – 1.9) | 0.291 |

Abbreviations: NK, natural killer.

**P742**

**The new system for the quality control in HPC units before the re-infusion in autologous transplant**

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Background: In our Center, the cell viability, the integrity of the bag and the clonogenic assay were evaluated before the reinfusion of HPC-A. This quality control should be made 14 days before the reinfusion to have the result of the functional test on the proliferative capacity of haematopoietic progenitors. Therefore, this study was designed to assess the potential of the Nucleocounter NC-3000 (Chemometec, Denmark) in our clinical routine.
Methods: The study was performed on 62 vials control that were carried out on HPC-A from 40 patients with various onco-hematologic malignancies. The number of total and viable CD34+ cells and viability were evaluated using 1 mL of thawed samples taken after DMSO washing. Viability was measured by the ISHAGE guidelines. The proliferative potential was assessed by specific clonogenic tests using a commercial medium. Furthermore, we evaluated the cellular functionality with NucleoCounter, by using two protocols: Vitality assay (analysis of the level of cellular thiols-GSH) and Mitochondrial potential assay (JC-1).
Results: The viability evaluated through flow cytometry of CD45 +/7AAD- and CD45 +/CD34 +/7AAD-are of 58% (23.69-92.38, SD 17.88) and 82% (5.14-100, SD 22.63) respectively. The evaluation of cells in pre-apoptosis measured by JC-1 assay showed a negative correlation (r = -0.40) with the total number of colonies obtained after seeding of 50.000/ml viable cells. We observed a statistically significant difference (p=0.009) comparing the median number of colonies (187.15&±SD 157.27) obtained with a value of JC-1 <30% to the number of colonies (41.77±SD 68.42) obtained with a value of JC-1>30%. No significantly difference was observed between the median number of colonies obtained and the analysis of the level of cellular thiols. Moreover the comparison between the flow cytometry and functional assays with NucleoCounter showed a negative correlation between the percentage of cells CD45+/CD34+/7AAD- and the percentage of JC-1 (r = -0.78).
Conclusions: The evaluation of cell functionality by the use of NucleoCounter device is in agreement with results from clonogenic assay and the NucleoCounter can be considered an effective alternative in the routine laboratory. In addition, this device represents a rapid screening method for evaluating the cryopreserved products at different times after their freezing.

**P743**

**Immunomagnetic selection in poor-mobiliser donors: use of new system for the optimisation of selection procedures**

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Background: Immunomagnetic cell selection (ICS) of CD34+ cells is increasingly used in allogeneic hematopoietic stem cell transplantation in order to reduce the T cells quantity. The aim of this study was to evaluate an automated protocol based on Ficoll method before ICS.
Study Design and Methods: The automated method was compared with the standard procedure. In the group 1 the cell processing involves the extraction of the buffy coat by Ficoll before incubation with antibodies. This procedure was performed with the Sepax S-100 device. The efficacy of this automated procedure was compared with the group 2. In this group, the cell washing and the incubation with antibodies were performed through the standard method. CD34+ cells from leukapheresis harvests were selected with ICS.
Results: The results obtained after the pre-incubation Ficoll cycle performed through the automated system Sepax S100 showed a total nucleated cells (TNC) and CD34+ cells recovery of 85.73% (range 75.90-90.63; SD 4.25) and 79.31% (range 51.77-112.31; SD 18.40), respectively. The TNC and CD34+ cells recovery after the pre-incubation washing cycle performed through the standard method, was 75.54% (range 38.36-97.76; SD 22.5) and 61,51% (range 30.87-81.79; SD 19.3), respectively.

The CD34+ cells recovery after ICS was 79% (range 51.77-100; SD 18.40) and 48.89% (15.57-88.24; SD 25.91) in the group 1 and the group 2, respectively. This difference was statistically significant (p=0.004).
Conclusion: The efficacy of the ICS led to an optimal purity without affecting the cell recovery, which resulted to be higher in the group 1. Overall, our data suggest that the Ficoll procedure for HPC-A concentrates before incubation of CD34+ is suitable for the clinical routine in the immunomagnetic cell selection for haploidentical stem cell transplantation in thalassemic patients.

**P744**
**Haematopoietic progenitor cells assessment for optimisation of peripheral blood progenitor cells harvest**
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Background: High dose chemotherapy with stem cell rescue is a well established treatment modality for some hematological malignancies. The number of CD34+ cells correlates with the leukapheresis CD34+ count and is a guide to the optimal timing to harvest peripheral blood progenitor cells (PBPC). At present, the determination of CD34+ cells is performed by flow cytometry, however the method is time consuming and economically demanding. Detection of hematopoietic progenitor cells (HPC) using hematology analyzer Sysmex XE-2100 is a quick and inexpensive test which is available as a part of complete blood count. HPCs are immature myeloid cells that are identified according to the cell size and their inner density. We evaluated whether HPC count could refine the prediction of PBPC harvest beginning.
Methods: From August 2007 to November 2011 we examined 133 persons before PBPC harvest (73 males, 60 females, median 59 years, range 19-68) suffering from multiple myeloma (n=79), lymphoma (n=139), acute leukemia (n=1), solid tumor (n=2), and also relative donors (n=12). In first 54 patients we examined the HPC count in the peripheral blood (PB) before the collection and in the collection bag, in next 79 patients only the HPC count in PB before the collection. We correlated the amount of HPC with the CD34+ cells count using Spearman rank correlation coefficient to find the minimum count of HPC for the optimal harvest beginning.
Results: The correlation of CD34+ cells *vs.* HPC (R=0.646, n=405, CI=95%) in PB was very good. However, the correlation of CD34+cells *vs.* HPC (R=0.244, n=254; CI=95%) in collection bag was very low. The HPC count 30/µl (sensitivity 92.3%, specificity 60.3%) in PB may be considered for the minimum to start the PBPC collection. The HPC count 10/µl (high sensitivity - 96.9%) in PB correlates with very low count level of CD 34 cells and may be the limit to exclude the collection. We established an algorithm to guide PBPC harvest beginning in our center. We begin with leukapheresis when the HPC count exceeded 30/µl.
Conclusions: Enumeration of HPC appears to be very fast and an acceptable tool for the improvement of PBPC harvest. Our data show a close correlation between HPC and CD 34 cell counts in accord with some other reports. However, the CD 34+ cells remain a useful predictor for both the PBPC harvest timing and successful PBPC collection.

**P745**
**CD3/C19 depleted PBSC for paediatric haploidentical transplantation can be conveniently processed and cryopreserved in advance of the conditioning without compromise in engraftment**
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Haploidentical hematopoietic stem cell transplantation is becoming a practical and realistic alternative to unrelated donor transplantation. In an effort to hasten immune reconstitution and reduce mortality from viral related complications, the Tubingen transplant group has pioneered the CD3/CD19 depletion procedure which is now practiced in several centers worldwide including Oman. The current technique involves the coordination of the conditioning, the PBSC collection and depletion procedure such that the final product is ready for infusion on the scheduled transplant day. In view of the relatively short shelf life of the antibodies and due to logistical reasons of order and supply of reagents in Oman, we were faced with the possibility of the expiry of expensive antibodies at a significant cost to the institution in case the trans-

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| | PATIENT 1 | PATIENT 2 |
|---|---|---|
| Diagnosis | HLH | HLH |
| Age | 2.5 yrs | 10 wks |
| Weight | 13.2 Kg | 4.4 Kg |
| Pre CRYO viability | 82% | 85% |
| Post THAW viability | 75% | 80% |
| Final CD34 (x $10^6$/kg) | 6.4 | 12.9 |
| Final CD3 (x $10^5$/kg) | 0.5 | 0.7 |
| Final CD19 (x $10^5$/kg) | 0.4 | 0.23 |
| Final CD56 (x $10^6$/kg) | 272 | 190 |
| ANC >0.5 x $10^9$/l | d+12 | d+14 |

plant had to be delayed. In order to circumvent this problem we decided to perform the PBSC and depletion procedure in advance of the conditioning and cryopreserve the final product until ready for use. Between July 2011 and November 2011 we performed two CD3/CD19 depleted haploidentical transplants. The results are shown in Table 1. For both patients the CliniMACS immunomagnetic cell separation system was used for the depletion procedures. For patient 2, the thawed product was washed with a dextran 40/albumin prior to infusion. Our results show that cryopreserved CD3/CD19 depleted PBSC can be used safely in pediatric patients undergoing haploidentical transplants with good results. Cryopreservation has two potential advantages. In general it avoids the need for specially timing this highly coordinated procedure with the conditioning such that it can be done at a time which is convenient for all the laboratory staff concerned. Secondly it could prove cost effective by avoiding the possible loss of expensive antibodies at risk for expiry.

**P746**

**Depletion of naïve lymphocytes with Fas-ligand *ex vivo* prevents graft-versus-host disease while maintaining T-cell support of engraftment and graft-versus-tumour activity**

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Graft versus host disease (GvHD) can be prevented by Fas-mediated selective depletion of host-sensitized donor lymphocytes *ex vivo*. This finding, together with the known trophic effects that Fas-ligand (FasL) exerts on hematopoietic stem cells, makes FasL treatment an attractive tool for graft engineering to enhance engraftment while reducing the risk of GvHD in patients. Host sensitization of allografts is, unfortunately, a laborious and technically challenging procedure that is likely to have limited appeal in the clinical setting. We tested the hypothesis that brief incubation of lymphocytes with recombinant FasL in the absence of host-specific antigenic stimulation can also alleviate GvHD. Brief exposure (24 hours) of unstimulated donor lymphocytes to recombinant FasL *ex vivo* results in balanced apoptosis of CD8+ and CD4+ subsets, while maintaining sustained responses to mitogenic stimulation. Infusion of lymphocytes so treated in the context of haploidentical transplant under conditions conducive to the development of severe GvHD resulted in attenuated weight loss following transplant as compared to mice infused with untreated lymphocytes; the clinical and histological score of skin and gastrointestinal GvHD were similarly improved following FasL treated T cell-replete haploidentical stem cell transplantation as compared to controls. Fatal GvHD was not precipitated by subsequent administration of LPS to mice after transplant of FasL treated lymphocytes, as opposed to high mortality rates in control animals. Although FasL-resistant donor T cells that survived the *ex vivo* incubation are less potent effectors of GvHD, they continued to facilitate hematopoietic progenitor engraftment, retained responsiveness to third party antigens, and were capable of elaborating graft versus tumor reactions. These findings in a preclinical model using a one-step culture procedure that can be easily performed in most clinical laboratories suggest that brief *ex vivo* incubation of hematopoietic grafts with FasL may improve the outcome and safety of clinical allogeneic and haploidentical transplants, abrogating the need for physical T-cell depletion.

**P747**

**Cryopreserved allogeneic peripheral blood stem cells result in outcome equivalent to those of fresh infusions enabling rational scheduling of donations**

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Introduction: Donor stem cells are traditionally infused fresh into recipients in the setting of allogeneic hematopoietic stem cell transplantation. We investigated outcomes of 133 sibling allografts using cryopreserved peripheral blood stem cells. Cryopreservation had no impact on engraftment when compared to data from registry studies. Nonrelapse mortality (NRM) and overall survival (OS) were within acceptable limits.
Methods: We identified recipients of HLA-matched sibling peripheral stem cells cryopreserved for a minimum of 7 days, who underwent allo-SCT at Hammersmith Hospital from 1998 until 2011(n=133). Thirty-five (26%) were transplanted for CML, 42(31%) for AML, 11(8%) for ALL, 14(11%) for myeloma and 13(10%) for other causes. Fifty-six (42%) had myeloablative and 77(58%) had reduced intensity conditioning. Using validated institutional protocols hematopoietic progenitor cell collections were cryopreserved the same day after collection or on following morning. Median CD-34+ cell dose infused was 9.83 $\times 10^6$/kg (range 2.4–33$\times 10^6$/kg). Engraftment was defined as a peripheral absolute neutrophil count (ANC) of 0.5$\times 10^9$/L for 2 successive days and platelet count of >50$\times 10^9$/L for 2 consecutive days, both without support.
Results: Overall 125(93%) achieved neutrophil engraftment and median time to engraftment was 19(range 10-42) days. Delayed neutrophil engraftment (>30 days) was present in 4 patients. Cumulative probability of achieving ANC> of 0.5$\times 10^9$/L for the whole cohort was 94%(88-95). One hundred and thirteen patients (84%) recovered platelets to >50$\times 10^9$/L within a median time of 21 (range 0-240) days. The cumulative probability of achieving platelets of 50$\times 10^9$/L was 84% (77–88). There was no association between CD-34+ cell doses infused and delayed or non engraftment of platelets. The incidence of acute

**[P747] Table 1. Comparison between Hammersmith (cryopreserved) and studies with fresh PBSC infusions**

| Study | Number of patiens | ANC to 0.5 $\times 10^9$/l | Platelets to 50 $\times 10^9$/l | Reference |
|---|---|---|---|---|
| Hammersmith | 133 | 19(10-42) | 21(0-180) | |
| EBMT | 288 | 14(10-40)[a] | 19(11-70) | Champlin et al, Blood 2000 |
| Seattle | 81 | 16(11-29) | NA | Bensinger et al, NEJM 2001 |
| Canadian | 109 | 19(12-35) | NA | Couban et al, Blood 2000 |

a-regular use of G-CSF

GvHD was 44% with grade II-IV GvHD 31%. The incidence of chronic GvHD was 30%, 50% of which was extensive. The day 100 NRM was 23% and OS at 3 years was 50%.
Conclusion: This study provides evidence that cryopreservation and subsequent infusion of peripheral blood stem cell harvests is safe and ensures durable engraftment, which is comparable to fresh stem cell infusions. We do not routinely use growth factors to aid count recovery and as such the time to engraftment data is consistent. Cryopreservation most importantly allows for flexibility in arranging admissions and scheduling conditioning regimens in busy units.

**P748**

**AML-reactive CD8+ cytotoxic T-lymphocytes effectively combat primary AML blasts in humanised NOD/SCID/ IL2Rgc-null mice**

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Introduction: We recently established an immunodeficient NOD/SCID/IL2Rgc-null (NSG) mouse model that allows reliable engraftment of human primary acute myeloid leukemia (AML) blasts, particularly those with high-risk FLT3-ITD mutations. Here we used these mice to analyze the anti-leukemia effect and homeostasis of human AML-reactive cytotoxic T lymphocytes (CTL) upon adoptive transfer *in vivo*. CTL were generated *in vitro* by stimulating healthy donor CD8+ T cells with FLT3-ITD+ AML blasts that were either HLA-identical to the donor or carried a single HLA class I mismatch antigen.
Methods: 5x10e5 AML blasts were injected into 6-8 week-old irradiated (150cGy) NSG mice to achieve >1% AML engraftment in bone marrow (BM) within 18 d resembling minimal residual disease. Subsequently, 5x10e6 alloreactive CD8+ CTL expanded over 14, 21, 28, and 56 d *in vitro* were transfused into these mice. Controls consisted of mice receiving leukemia alone or AML and CTL of irrelevant antiviral specificity. Mice were supplemented with human interleukin (IL)-2, IL7-Fc, and additionally IL-15 upon transfer of HLA-identical T cells. AML infiltration was analyzed in kinetic studies 2 h, 24 h, 48 h, 7 d and 28 d after CTL transfer.
Results: We observed complete eradication of FLT3-ITD+ AML blasts in BM, spleen and peripheral blood of mice one week after transfer of single HLA-mismatched CTL cultured for 14, 21, or 28 d, respectively. In contrast, control mice showed 25-61% (median, 35%) leukemia infiltration in BM. Kinetic analysis demonstrated almost complete AML remission as early as 48 h after T cell transfer, and no relapse was detected for up to 42 d. *Ex vivo* analysis of CTL re-isolated 24 and 48 h after injection showed persistent reactivity to AML blasts, but not to NSG-derived murine dendritic cells. Interestingly, CTL expanded over 56 d *in vitro* appeared less capable to eradicate AML *in vivo*. Results were reproducible in 2 different donor-patient pairs. Moreover, significant reduction of leukemia infiltration could also be observed in mice that had received HLA-matched AML-reactive CTL (n=2). Further experiments on kinetics, T cell dose and repetitive injections are currently ongoing.
Conclusion: We show herein that NSG mice engrafted with primary human AML blasts can be successfully treated with human alloreactive CTL. The model will be further optimized to serve as a general platform for testing the anti-leukemia effect of T cell grafts before adoptive transfer into humans.

**P749**

**Volume reduction and complete red blood cell depletion of bone marrow harvest for haematopoietic stem cell transplantation with a new processing system, Sepax 2**

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Objectives: During bone marrow harvesting used for for hematopoietic stem cell transplantation (HSCT) large volumes of bone marrow are collected. To minimize the risk of ABO-induced transfusion reactions, volume overload during marrow transfusion and direct hemoglobin toxicity in case of the use of cryopreserved units, a volume reduction with red blood cell (RBC) depletion may be indicated. During each processing step it is critical to maintain an optimal CD34+ cell recovery. We present preliminary data of the new fully-automated Sepax 2 (Biosafe, Eysins, Switzerland) cell separation device, which has a versatile platform designed for efficient processing of bone marrow.
Methods: A two step approach was designed: first a volume reduction to 50-150ml per maximal 880 mL initial bone marrow volume and secondly a density gradient based sedimentation to eliminate the residual RBCs. During this pilot phase only the surplus of the harvest (surplus of 2x10e8 total nucleated cell (TNC)/kg)) was processed. We evaluated TNC, CD34+ cells, and yield before and after each step, as well as the residual RBCs at the end of the procedure.
Results: Within the last 6 months, we processed 4 bone marrow units. The volume processed ranged between 185 mL and 692 mL. The results of the volume reduction and RBC depletion are listed in Table 1. In all four processed bone marrow units, the RBC depletion was effective, reaching values below the fixed cut-off of 15ml needed in case of major ABO barrier. The CD34+ yield was diverse: two procedures ended with a yield of 90% or more, and two others with a yield of 32 and 37% respectively. The reason for this variability is not yet clear. In one case (BS 9511) it could be due to a bone marrow of an aged donor (over 65 year) with a high fat content. Volume reduction achieved good results in respect of CD34+ yield in all cases. In one case of unsatisfactory CD34 recovery (BS 8411) the rescue by re- processing the waste bag was tested: we could extract an absolute CD34+ cell count of 105.7x10e6.
Discussion: Our preliminary results with the new Sepax 2 system look promising, approaching the results obtained with the standard methods of bone marrow processing with COBE Spectra or Amicus. This new method has the advantages to be fully automated and working as a closed system, which is mandatory to conform to the JACIE criteria of stem cell engineering.

**P750**

**The new cord blood processing system, Sepax 2 provides higher recovery of total nuclear counts after volume reduction**

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Objectives: Cord blood unit (CBU) volume reduction performed prior to freezing is an accepted and useful method to reduce

**[P749]**

Table 1.

| | Before processing | | | | After Volume Reduction | | | After RBC Depletion | | | | Recovery | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vol (ml) | TNC abs (x10e8) | RBC Vol (ml) | CD34+ abs (x10e6) | Volume (ml) | TNC abs (x10e8) | CD34+ abs (x10e6) | Volume (ml) | TNC abs (x10e8) | CD34+ abs (x10e6) | RBC Vol (ml) | TNC (%) | CD 34+ (%) | RBC (%) |
| BS8411 | 689 | 23.22 | 180.3 | 238.8 | 60 | 21.0 | 273.7 | 109 | 1.33 | 77.96 | 0.7 | 5.7 | 32.6 | 0.2 |
| FRifi1404 | 185 | 3.66 | 49.6 | 29.44 | 64 | 2.71 | 32.7 | 46 | 1.53 | 32.82 | 0.4 | 41.8 | 111.5 | 0.7 |
| BS9511 | 692 | 17.02 | 224.2 | 174 | 120 | 11.24 | 93.2 | 49 | 4.64 | 64.9 | 1.4 | 35.9 | 37.3 | 0.7 |
| BS9611 | 633 | 7.34 | 198.8 | 53.19 | 125 | 5.54 | 49.54 | 46 | 3.71 | 48.12 | 10.1 | 50.6 | 90.5 | 5.0 |

**[P750]**

Table 1: Comparison of cell processing results between Sepax 1 and Sepax 2

| | Sepax 1 (n=195) | | | Sepax 2 (n= 110) | | |
|---|---|---|---|---|---|---|
| | At collection Median (range) | After Processing Median (range) | Recovery Median (%) | At collection Median (range) | After processing Median (range) | Recovery Median (%) |
| Volume (ml) | 111.0 (71-196) | | | 116 (75-191) | | |
| TNC (x10e7) | 134.9 (74.3 - 391.2) | 101.2 (55.7- 263.2) | 75.9 (40.0-94.6) | 135.5 (91.2-389.8) | 103.6 (68.8-300.8) | 76.9 (63.1- 91.0) |
| CD34 (x10e6) | 3.6 (0.9 - 20.3) | 2.9 (0.7 - 15.8) | 82.6 (45.2-160) | 4.1 (1.0-19.6) | 3.5 (0.6 -17.3) | 84.8 (54.9- 113) |

storage space. Since the year 2000 we use in our cell processing unit the fully-automated Sepax® (Biosafe, Eysins, Switzerland) cell separation device. The new device (Sepax® 2) is also a fully-automated system with a versatile platform designed for efficient processing of umbilical cord blood, bone marrow and other cellular material. The aim of the study was to compare the efficacy of a new device, compared to the previous system for CBU volume reduction.
Methods: In the Cord-Blood Bank Basel 110 CBU were processed with the new device from July 14th until end of October 2011. The results were compared with 195 processed CBU performed between January and July 13th 2011 with the previous Processing System. Following parameters were evaluated: total nucleated cell counts (TNC), absolute viable CD34+ cells at collection/before cryopreservation as well as recovery rates (Yields). According to the new rules of Swisscord (established since November 1st 2011) only CBU with a minimal TNC of 150 x10e7 at collection are processed. Thus, we were especially interested in the efficacy of recovery in cord blood with high TNC counts.
Results: There was no statistical difference between the two systems for TNC and CD34 counts before and after collection and no difference in overall recovery (Table 1). We compared a subgroup of the CBU with TNC counts ≥150x10e7 at collection (66 CBU with Sepax1; 45 with Sepax2). With the new device we found a significant higher TNC count after processing (median 136.2 x10e7 (range, 70.-263.2) *vs* 146.0x10e7 (111.6-300.8; p 0.015) and an improved TNC-recovery (median 75.4%; range 40.0-91.3; *vs* 77.59%; range 63.3-86.9; p 0.029) as well (Figure 1).
Conclusion: Overall both devices showed good TNC, CD34 counts after volume reduction and excellent recovery rates of TNC and CD34. In a subgroup of CBU with a higher TNC at collection the Sepax 2 yielded better TNC counts and an improved TNC-recovery. Considering that Cord Blood Banks concentrate increasingly their collection activity on CBU with high cellular counts, this finding could contribute to achieve this goal.

**P751**
**New GMP-grade, xeno-component free medium for activation and expansion of T-cells**
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Therapeutic applications of T cells in immunotherapy have recently gained momentum with the promising results in adoptive transfer of antigen-specific T cells for infectious complications after allogeneic stem cell or solid organ transplantation or for immunotherapy of malignant diseases. Activation and expansion of these cells for clinical application under controlled conditions require GMP-grade reagents including appropriate antibodies, cytokines and media. For standardized, reproducible cell cultivation and *ex vivo* differentiation procedures, a new serum and xeno-component free, GMP-grade medium for clinical use has been developed. High lot-to-lot consistency has been achieved by eliminating protein components not relevant for T cell expansion leaving human serum albumin as the only protein component.
Using soluble antibodies against CD3 and CD28, more than 30%-higher expansion rates of viable and functional T cells after 6 days of expansion have been achieved with the new xeno-component free medium compared with other serum-free media. Transferring the same protocol to a high density cell culture system such as a gas permeable rapid expansion device, high densities of T cells with more than $1.5x10^7$ cells/ mL were reached.
The generation of antigen-specific T cells using the Cytokine Capture System IFN-gamma and the serum and xeno-component free T cell medium showed similar results regarding purity, recovery and background stimulation compared to the use of a standard basal medium supplemented with 10% human AB serum. For the automation of such complex procedures, a new cell processing device was developed. All steps for the antigen-specific T cell processing, i.e. antigen-specific re-stimulation, magnetic enrichment, and *in vitro* expansion with this T cell medium are performed in this fully automated device, in a closed system under sterile conditions.
In conclusion, the newly developed GMP-grade, serum and xeno-component free T cell medium demonstrated high lot-to-lot consistency and was superior in its performance to other commercially available serum-free media in high density cell culture systems. The new medium can be used to replace human AB serum supplementation for the clinical manufacturing of T cells resulting in easier handling and higher consistency.

**P752**
**Fully automated cell separation system for clinical scale separation of CD133+ cells from bone marrow aspirates**
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An automated clinical-grade process to separate CD133+ cells from human bone marrow aspirates (BM) was developed. The separated cells are intended to be used for cellular therapies in the field of regenerative medicine such as cardiovascular, peripheral artery, and liver disease. The process is intended to fulfill all requirements for an automated sample preparation and separation of CD133+ cells from human BM. To determine the performance of the fully automated separation process, CD133+ cells were separated from sternal bone marrow. The initial frequency of CD133+ cells amounted to 0.34% (range: 0.1% to 0.7%) and the number of isolated CD133+ cells was $7.3x10^5$ (range: $3.7x10^5$ to $1.9x10^6$). The yield was 53% (range: 18.6% to 90%) and the average viability of the separated CD133+ cells achieved 86% (range: 70% to 97%). The depletion of CD133 negative cells was greater than 99.9%. To demonstrate cell functionality colony-forming unit assays were performed to evaluate the differentiation potential of hematopoietic stem and progenitor cells. As expected, the CD133+

fraction contained primitive and multipotent progenitor cells. An important determinant of successful stem cell transplantation is the ability of transplanted cells to mobilize, home, migrate, and efficiently engraft and repair damaged tissues with functional cells. The chemokine SDF-1alpha plays a central role as a chemoattractant for CD133+ stem and progenitor cells, regulating their motility, homing to, and retention, survival, and proliferation in the BM. To determine the motility of enriched CD133+ cells to SDF-1alpha, transmigration assays were performed. First results showed that 43% of CD133+ cells, separated from bone marrow aspirates, migrated to SDF-1alpha. Without SDF- 1alpha, 0.7% of CD133+ cells migrated. At the end of the process the enriched CD133+ cells were available in around 6 mL sodium chloride solution (NaCl). To provide a more physiological environment for CD133+ cells the isotonic NaCl can be optionally automatically supplemented with 10% autologous plasma for storage or transport of cells. The described cell separation system provides an efficient and convenient way to purify CD133+ cells from BM within 2.5 h without any intermediate manual steps. The cell preparation in a closed, sterile system facilitates a fast and robust enrichment of CD133+ cells. After separation the CD133+ cells are eluted in a small volume and can immediately be used for further applications.

**P753**

**Fully automated generation of multi-virus-specific CD4+ and CD8+ T-cells for adoptive immunotherapy**

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Adoptive transfer of virus-specific T-lymphocytes can be a powerful strategy to treat or prevent opportunistic infections in immunocompromised patients. Human adenovirus, Epstein-Barr-virus, and cytomegalovirus infections are frequently observed and often cause life-threatening complications following allogeneic stem cell transplantation. Multi-virus-specific CD4+ and CD8+ T-cells can rapidly be generated by a short-term antigen-specific restimulation of peripheral blood cells with a combination of pools of peptides covering complete viral antigens and a subsequent isolation using the Cytokine Capture System IFN-gamma. A novel cell processing device was developed, which performs all steps of the CCS procedure, i.e. restimulation, magnetic enrichment, and potentially *in vitro* expansion fully automated under sterile conditions. All components for the generation of the cellular product including the cellular starting material (e.g. leukapheresis), antigen(s), reagents, buffer, and media are connected to a sterile single-use functionally closed tubing set via sterile filters or docking technique. Cell processing can run overnight and the isolated cells might be used directly after magnetic enrichment or after an additional phase of *in vitro* expansion. Using this cell processing device, IFN-gamma secreting multi-virus-specific T-cells can be enriched to the same purity as with the semi-automated procedure and cell loss is markedly reduced, leading to an increased yield of IFN-gamma positive cells. Furthermore, observing an improved cell viability using the automated compared to the semi-automated process, resulting in better *in vitro* T cell expansion rates. Restimulation of isolated and expanded multi-virus-specific T cells with an antigen mixture resulted in a high proportion of T-cells re-expressing IFN-gamma, confirming their functionality. Analysis of the composition of the multi-virus-specific T-cell population in respect to single antigen specificities revealed the relative frequencies of T-cells specific for each single antigen were comparable before and after the co-enrichment and co-expansion process, showing the protocol works for T-cells independent of their pathogen specificity. In conclusion, the automation enables with minimal manual intervention, reduced workload and also clean room requirements the easy, safe, fast, robust generation of antigen-specific T-cells for adoptive immunotherapy.

**P754**

**CD34(+) cell selection and purging in haematopoietic stem cell transplantation: technical report of a single centre**

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Background: CD34 positive selection has been succesfully used to deplete tumor cells from grafts for autologous transplantation, to deplete T cells for allogeneic transplantation for 15 years. The first CD34 positive selection in Turkey was carried out in Gulhane Military Medical Academy Bone Marrow Transplantation (BMT) center in February 1998 using immunomagnetic method (CliniMACS).
Methods: One hundred sixty-eight selection processes have done until October 2011. Sixty-eight and one hundred selections were made for autologous transplantation and for haploidentical transplantation, respectively . Of the 68 autologous transplantation process, number of diagnosis were as follows: 29 breast cancer, 13 Non-hodgkin lymphoma, 15 neuroblastoma, 2 Acute Lymphoblastic Leukemia, 1 scleroderma, 1 multiple sclerosis, 4 Acute Myeloid Leukemia, 2 multiple myeloma and 1 osteosarcoma. Among the 100 process of haploidentical allogeneic transplantation, 53 Severe Combined Immunodeficiency, 13 Fanconi anemia, 5 osteopetrosis, 17 Acute Myeloid Leukemia, 4 Acute Lymphoblastic Leukemia, 1 Chronic Myeloid Leukemia, 1 Chronic Myeloid Monocytic Leukemia, 1 MHC Class II deficiency, 1 Myelodysplastic Syndrome, 1 hemophagocytic syndrome, 2 amegacaryoctic thrombocytopenia and 1 Hemophagocytic Lymphohistiocytosis Syndrome were identified. Normal column was used in 35 selection and large column was used in 133 selection. Peripheral blood was used in 159 selection and bone marrow in 9 selection. Manual washing was used for 69 cases and automatic washing for 99 cases.
Results: The median rate of purity and recovery ratio of CD 34 (+) cells were 94% (30-99) and 66% (27-100), respectively. The median T-cell depletion was 4:52 log (1.31-5.82) and B-cell depletion was 2.86 log (1.11-4.82). For the > 94 purity rate, tumor cell depletion was between 3-4 log. Selections administered in healthy subjects had higher purity and recovery rates than in patients. The performance of the system was not compromised even though the number of cells were higher than the capacity (provided the total column CD34 (+) cell capacity of CliniMACS is not exceeded).
Conclusion: In Conclusion, immunomagnetic selection of CD34 (+) cells using CliniMACS is an effective method for both autologous and allogeneic transplantation, providing high purity and protection rates of CD34 and high depletion rates of T and B cells.

**P755**

**Donor lymphocyte collections using the Spectra Optia MNC version 5**

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We report our preliminary experience on the use of the Optia MNC version 5 for collection of donor lymphocytes from normal donors for use in donor lymphocyte infusions (DLI) in allogeneic transplantation.
Methods and Materials: 10 non-mobilized allogeneic donors have had DLI apheresis collections on Optia MNC version 5. Collection preference was set to deliver a low haematocrit final product. ACD-A anticoagulation was used and apheresis completed at an AC ratio of 13.5 and infusion rate of 0.8mL/min/L total blood volume (TBV). Run data were recorded collected from the Spectra OptiaTM apheresis reporting system at the end of each procedure. Full blood count

[P755] Table 1.

| | Whole blood processed (mL) | Procedure time (minutes) | Total collect volume (mL) | Product RBC volume (mL) | GRN % | CD3+ CE1% | CD3+ dose/ kg | PLT reduction pre to post apheresis (%) |
|---|---|---|---|---|---|---|---|---|
| median | 11144 | 265 | 143 | 2.9 | 1.50 % | 68.80 % | 1.29x10^8 | 35.60% |
| max | 21254 | 415 | 198 | 7.3 | 24.10% | 85.60 % | 2.85x10^8 | 52.10% |
| min | 8285 | 226 | 124 | 2 | 0.10 % | 36.00 % | 8.62x10^7 | 20.00% |

data and CD3+/CD19+ target cell numbers were completed for the donor before and after apheresis and from the final product.
Performance was measured by target cell yield and collection efficiency together with final product volume and haematocrit. Platelet loss to the donor was also measured after apheresis.
Results: Performance data are summarized in Table 1.
Conclusion: We conclude the Optia MNC version 5 delivers a high purity MNC product with low final volume. CD3+ target cells were collected with high overall efficiency, with an acceptable yield for escalating doses of DLI. Residual red cell volume and granulocyte numbers in the final product was low and platelet loss to the donor was acceptable.

## Minimal Residual Disease, Tolerance, Chimerism and Immune Reconstitution

**P756**
**Single-centre prospective feasibility study on prophylactic donor lymphocyte infusions in leukaemia patients transplanted with low-dose alemtuzumab**
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Since immunotherapy seems to be more effective in stages of impending, rather than overt relapse, perhaps the most appropriate platform for donor lymphocyte infusions (DLIs) is in a prophylactic setting. Fifty-seven patients with haematological malignancies received an allogeneic hematopoietic cell transplantation (HCT) with low dose (10-20 mg) alemtuzumab and were prospectively screened for prophylactic DLI (pDLI), if they had, high risk acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) or increasing mixed chimerism (MC). Patients had to be out of immunosuppression and not to have a history of acute GvHD≥ grade II or an active GvHD at time of the planned pDLI. Finally, 15 (37%) patients (AML 8, ALL 7) received a total of 47 pDLIs administrations. pDLIs from sibling donors (n=7) were given after second leukapheresis without growth factor mobilization, and from unrelated donors (n=8) from frozen aliquots taken from the mobilized peripheral blood stem cell (PBSCT) graft. At the time of first pDLI, 8 patients (53%) revealed MC (median 7.5%, 5-15). The first pDLI was given at a median of 162 days (78-426) after HCT, the median number of infusions was 3 (1-6) and the median cumulative dose given was $2x10^6$ CD3+ cells/kg (0.7-7). 75% pDLI recipients converted their MC to stable complete chimerism and 25% improved to ≤5%. 47% pDLI recipients developed graft-versus-host-disease (GvHD; acute GvHD n=4, chronic GvHD n=3) after a median cumulative CD3+ dose of $2x10^6$/kg (0.7-7) and median 75 (33-343) and 36 days (11-126) after the first and the last pDLI, respectively. After a median follow up of 529 days (374-1606) from HCT, 11 (73%) pDLI recipients are alive and 4 (27%) died, all due to GvHD related causes. None of the patients that received pDLIs relapsed. In contrast, 3 out of 7 (43%) of the patients who were screened as potential pDLI candidates but did not receive lymphocytes because of logistical hurdles relapsed. Taken together, our prospective feasibility study shows that pDLIs are feasible and probably maintain significant anti-leukemic activity, as suggested by the high chimerism conversion rate and the zero relapse rates. However, the high incidence of GvHD, even after low CD3+ dose, emphasizes the need for more careful selection of patients receiving pDLI or the use of safer innovative cellular products, such as suicide-gene engineered lymphocytes.

**P757**
**Fast immune recovery following unmanipulated haploidentical BMT with post-transplant high-dose cyclophosphamide as GvHD prophylaxis: a comparison with siblings, unrelated donors, cord blood**
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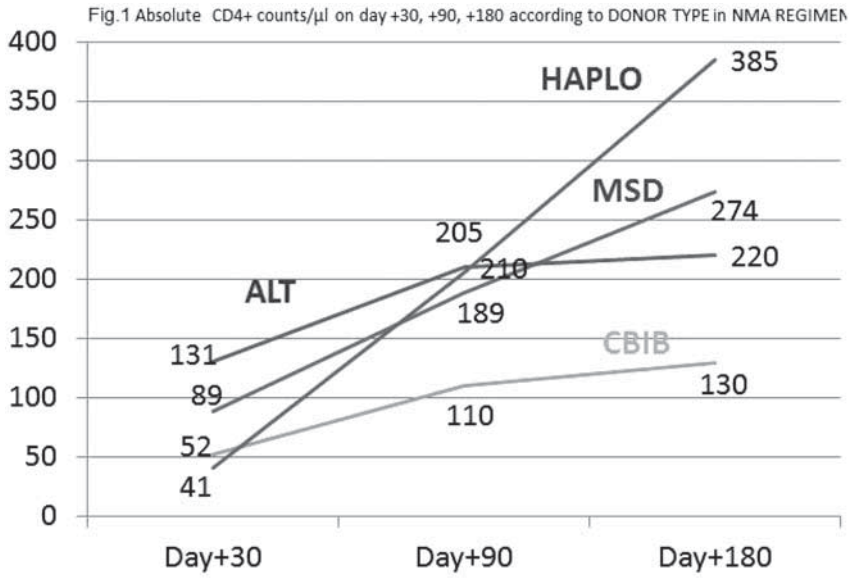
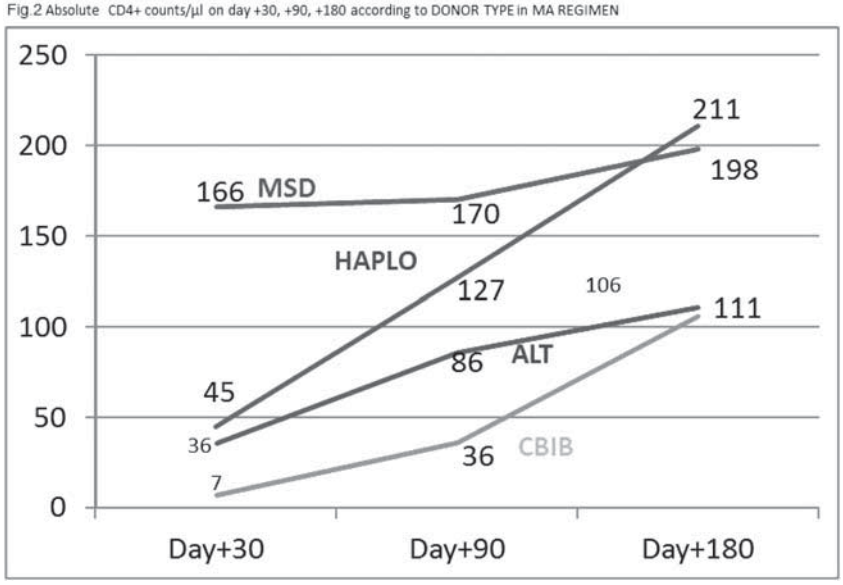
We report immune recovery after HSCT in 444 patients according to donor type: HLA identical Siblings (MSD) (n=176), 1 antigen mismatched family donors- unrelated donors (n=125) (ALT), unrelated cord blood (n=103) and haplo-identical mismatched family donors (HAPLO) (n=40).
Methods: All patients received unmanipulated bone marrow: 283 after a myeloablative (MA) conditioning regimen (CY-TBI or BU-CY) and 161 after a fludarabine based reduced intensity regimen (RIC). Graft versus host disease (GvHD) prophylaxis was cyclosporin methotrexate (CyA+MTX) for all patients except for CBIB (CyA and mycophenolate,MMF) and for HAPLO transplants which consisted of CyA+MMF and post-transplant high dose cyclophosphamide (HDCY). Anti-thymocyte globulin (ATG) was used only for ALT transplants. We compared T cell reconstitution (CD3+, CD4+ CD8+) and B cell recovery (IgA levels) separately after MA and RIC transplants, according to donor type, on days +30, +90, +180 post BMT.
Results: T cell recovery. As shown in Figure 1, following NMA regimens, CB transplants had the slowest CD4 recovery; HAPLO CD4 recovery was comparable to MSD until day +90, but exceeded all other donor types on day +180. Non-Relapse Mortality (CI-NRM) was 30% (MSD), 33% (ALT), 45% (CBIB), 0% (HAPLO) (p=0.02).

[P757]




Shown in Figure 2 are CD4 counts following a MA: MSD donors had best CD4 recovery, followed by HAPLO grafts, which were comparable on day +90 and +180. ALT donors had a slower recovery, especially on days +90 and +180, and CB transplants were again the slowest. Non-Relapse Mortality (CI-NRM) for MA regimens was respectively 18% (MSD), 35% (ALT), 34% (CBIB), 22% (HAPLO) (p=0.02).

Results B cell recovery. The level of serum IgA on days +90, +180 were comparable in MSD and HAPLO transplants (52 *vs* 39, p=0.7; and 53 *vs* 41, p=0.3). MSD had significantly higher IgA levels as compared to ALT grafts (52 *vs* 28, p=0.0005; and 53 *vs* 25, p=0.0005) and also when compared to CB grafts (52 *vs* 28, p=0.008 and 53 *vs* 30, p=0.01).

Conclusions. CD4 recovery and IgA serum levels after SCT are comparable in patients receiving MSD and HAPLO transplants. Immune reconstitution is significantly delayed in patients receiving grafts from unrelated donors, 1 antigen mismatched family members, or cord blood. In keeping with these results, faster immune recovery in HAPLO grafts with HDCY post-transplant, is associated with a low risk of lethal infections.

**P758**

**Monitoring of minimal residual disease among multiple myeloma patients after autologous stem cell transplantation**

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Objective: Multiparameter flow cytometry (MFC) immunophenotyping (IF) can be a sensitive method for analyzing the plasma cells (PCs) compartment in patients with multiple myeloma (MM), allows discrimination between myelomatous and normal PCs.

The aim of this study was evaluation of residual tumor cells in bone marrow by MFC.

Materials and Methods: In this study 6 patients with MM were screened, age 33-66 (M-55).

IF of bone marrow cells (BMC) was performed before stem cell mobilization (SCM) and after 2-6 months after autologous stem cell transplantation (ASCT). SCM included cyclophosphamide

4 g/m$^2$ plus G-CSF 5 µg/kg/day. Prior ASCT conditioning included melphalan 200 mg/m$^2$.
Quantification of neoplastic cells was perfomed by using Beckman Coulter FC-500 flow cytometer. IF studies were perfomed on erythrocyte-lysed BM aspirate samples: CD 138 FITC/CD 38 PE/CD 19 PerCP; CD 138 FITC/CD 38 PE/CD 117 PerCP (c-cit) Cy 5.5; CD 138 FITC/CD 38 PerCP Cy 5.5/CD 56 PE (monoclonal antibodies BD).
Minimal residual disease (MRD) was estimated according to abberant antigen expression (AAE)CD 19, CD 117, CD 56 from gate with coexpression CD 138/38. The analysis was based on at least 50,000 events. Threshold level of cells with abnormal antigen expression of CD 138/CD 38 population was 10%.
The IF results were compared with immunofixation and morphology studies.
Results: 1 patient achieved complete response (CR)before SCM, that was confirmed by immunofixation (IMF)and by morphology (0,8 % BMPCs) and was MRD (-) by MFC. 4 patients were in very good partial response (VGPR) (trace secretion in serum and/or urine M-protein and 0-1,2 % BMPCs), among 3 of them was MRD (+) by IF, in 1 case MRD was undetectable. Partial response (PR) was achieved in 1 patient (serum M-protein-13 g/l; 3,5 % BMPCs) and MRD (+) by IF.
IF of BMC among 4 patients after ASCT confirmed achievement of stringent complete response (sCR): absence of AAE CD 19, CD 117, CD 56 on cells with coexpression 138/38, absence of M-protein in serum and urine, 0-0,5 % PCs in BM. There were 1 patient in CR before SCM and 3 patients with VGPR. Another patient with VGPR before SCM still had the same data after ASCT: trace secretion in serum; 1,2 % BMPCs and MRD (+). Only 1 patient in PR after ASCT hasn't get responder rate: without having any changes in serum M-protein, he was otherwise MRD negative due to a few-celled BM.
Conclusions: IF BMC among MM patients by flow cytometry allows to identify CR after ASCT.

**P759**

## Post-transplant minimal residual disease by multiparameter flow cytometry strongly correlates with long-term outcome of patients with multiple myeloma undergoing autologous haematopoietic stem cell transplantation

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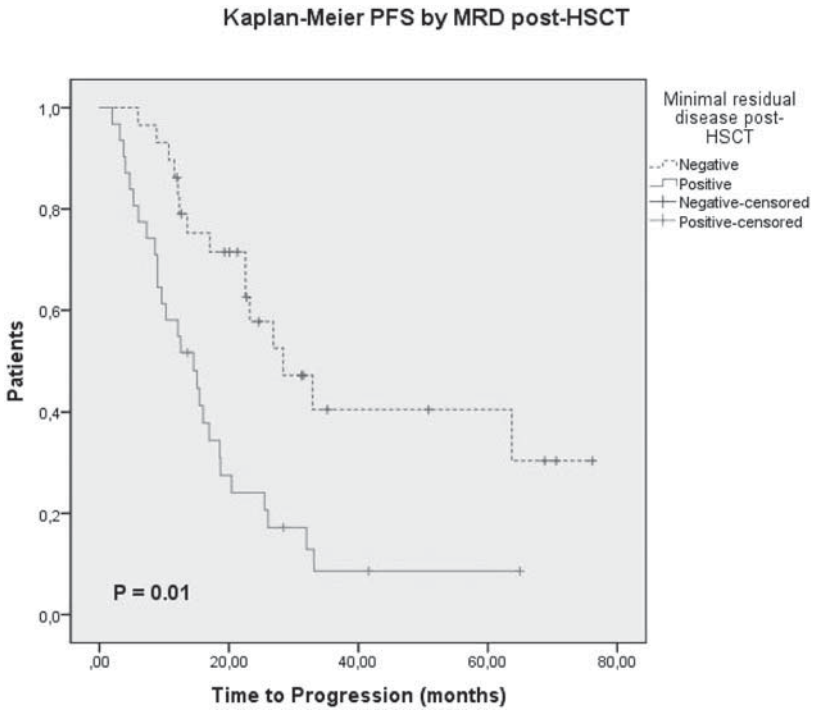
Objectives: Multiple Myeloma (MM) is a progressive and incurable disease with classical chemotherapy or high-dose therapy (HDT). However, the clinical course can be very variable, including patients very short progression-free survival, and patients with prolonged symptom-free survival. The development of new techniques for detecting minimal residual disease (MRD) has led to the need for further refinement of MM response criteria used so far. We evaluated the influence of the MRD detection by multiparameter flow cytometry (MFC) on progression-free survival (PFS) and treatment-free interval (TFI) in patients undergoing autologous hematopoietic stem cell transplantation (HSCT).
Patients and Methods: we retrospectively reviewed 76 patients treated according to the Spanish multicenter protocol GEM 2000: induction with 6 alternating cycles of VBCMP/VBAD followed by auto-HSCT conditioned high-dose melphalan. MRD status by MFC was determined pre-transplant and at day +100 post-transplant. Immunophenotyping was performed in bone marrow samples with a FACSCalibur flow cytometer (4-colours). For each sample ≥ $10^5$ cells were acquired. Results: MDR by MFC was negative [-] in 28% prior to HSCT as compared to 51% after transplantation. All patients who were MRD [-] pre-HSCT remained MDR [-] post-HSCT. PFS (median 28 *vs* 14 months, P=0.01), and TFI (median 28 *vs* 17 months, P=0.01) were longer in pts who were MRD [-] *vs* MRD positive [+] at day +100 after HSCT, when measured by MFC. Contrarily, using the classic response criteria by immunofixation (IFx [+] *vs* IFx [-]), no significant differences were found at day +100 after HSCT (PFS: median 23 *vs* 17 months, P=0.2; TFI: median 28 *vs* 20 months, P=0.2). Better outcomes were observed in patients who were already MRD [-] by MFC pre-HSCT com-

**[P759]**



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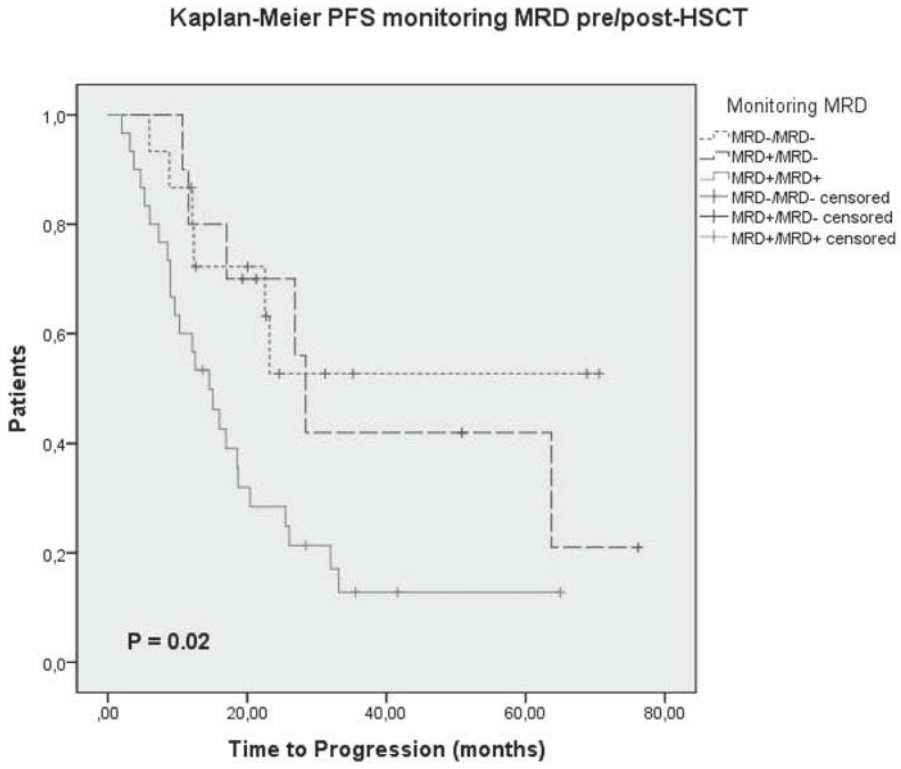


pared to those who reached the MDR [-] status post-HSCT. In multivariate analysis, MRD status by MFC at day +100 after HSCT was the most important independent prognostic factor for PFS (HR=6.7, P=0.007) and TFI (HR =6.1, P=0.005).
Conclusion: our findings confirm the importance of MRD monitoring by MFC in MM patients undergoing HSCT and supports the need to incorporate the technique in routine clinical practice. Particularly, the strong impact of the MRD status by MFC at day +100 on the future course of the disease, may help to decide what patients might benefit from consolidation therapies or early allogeneic transplant.

**P760**

**Phenotypic shift after allogeneic transplant: an open issue for minimal residual disease assessment in acute myeloid leukaemia**

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Background: The lack of effective therapies for the treatment of leukemia relapse after allogeneic transplant (HSCT) underlines the need to prevent relapse occurrence. Thus, the identification of reliable predictors of impending relapse would support a rational use of post HSCT procedures, such as reduction/ suspension of immunosuppression or donor leukocytes infusion (DLI), aimed to reduce or delay overt reoccurrence of leukemia.
Methods and Aims: The aim of our study was to evaluate the predictive power of MRD by flow cytometry (FC) on post-HSCT relapse prediction in patients with Acute Myeloid Leukemia (AML). Since February 2010 to September 2011, 9 AML patients with an available leukemia-associated aberrant immunophenotype received an allo-HSCT. Median age was 48 (range 16-59). Based on clinical- biological characteristics at diagnosis, 8 and 1 patients were classified as having high and intermediate risk disease, respectively. We prospectively studied the MRD levels by FC at days 30, 90, 180, 270 and 365 after HSCT.
Results: After a median follow-up of 13 months, 8 patients are in CCR. Two patients experienced relapse; one of them had a meaningful clinical and biologic behavior. The HSCT source was HLA-identical sibling donor; the patient had a complete peripheral recovery at day +30 in spite of a mixed chimerism status (20%). As his peripheral values declined at day +80, one BM aspirate was carried out: MRD by FC resulted negative. Due to blood count worsening, a further evaluation (day +120) showed BM infiltration by blast cells at morphology; at the same time, the patient experienced pleural effusion due to extra-hematological localization. As depicted in figure 1, the phenotypic profile of cells showed a complete shift from diagnosis to relapse with loss of CD34 and CD117 and with a tendency to monocytic differentiation that was not present at the outset. A donor origin of AML was ruled out; the biologic re-assessment showed normal karyotype, negativity for NPM1 and FLT3 mutations, as highlighted at diagnosis. The patient received a salvage therapy based on high doses cytarabine and died during aplasia without any available evaluation of response.
Conclusions: The occurrence of a phenotypic shift by blasts can be relatively frequent after allo-HSCT; it can impair significantly the sensitivity of MRD assessment by FC. A wide AML phenotypic characterization and the use of multiple combinations on its basis are crucial to avoid a relapse misdiagnosis.

**P761**

**WT1 transcript's kinetics after allogeneic transplant in acute myeloid leukaemia**

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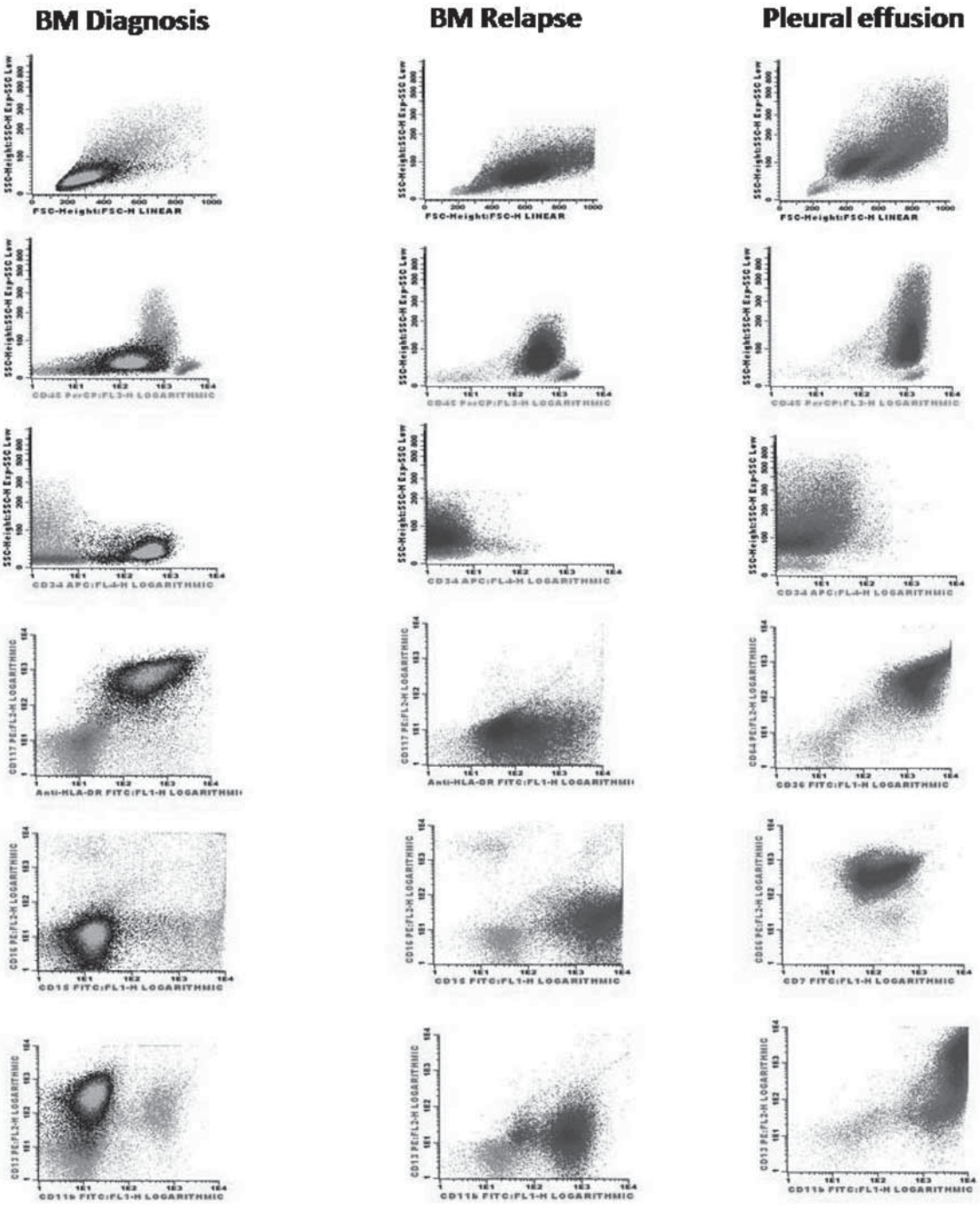
Background: The lack of effective therapies for the treatment of leukemia relapse after allogeneic transplant (HSCT) underlines

[P760]




the need to prevent relapse occurrence. Thus, the identification of reliable predictors of impending relapse would support a rational use of post HSCT procedures, such as the reduction/suspension of immunosuppression or donor leukocytes infusion (DLI), aimed to reduce or delay overt reoccurrence of leukemia.

Methods and Aims: The aim of our study was to evaluate the predictive power of WT1 transcript monitoring post-HSCT, in patients with Acute Myeloid Leukemia (AML), on relapse prediction. Since February 2010 to July 2011, 10 AML patients received HSCT while on first complete remission (CR); their median age was 48 (range 16-59). Based on clinical- biological characteristics at diagnosis, 9 and 1 patients were classified as having high and intermediate risk disease, respectively. We prospectively analyzed the patients at specific time points: day 30, 90, 180, 270 and 365 after HSCT. At each time-point, the expression of WT1 by RT-PCR on bone marrow was planned.

Results. Six out of 10 patients over-expressed WT1 at diagnosis and were therefore eligible for our study. In all these cases WT1 levels reduced below the adopted threshold of normality (250 WT1 copies per Abl copies) before HSCT. All patients are in continuous CR after a median follow-up of 13 months (range

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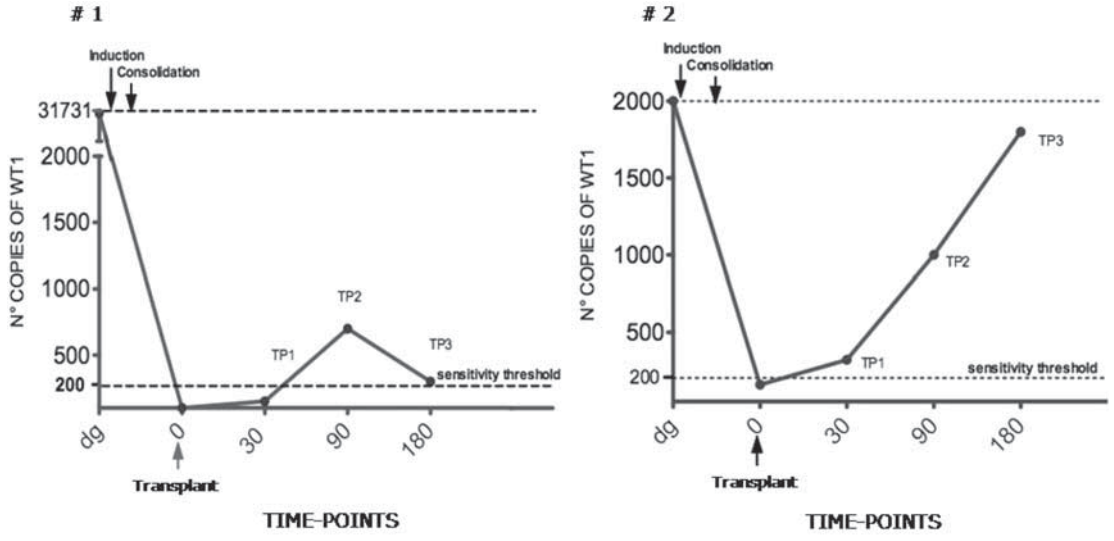


8-21). Four of them persist having normal levels of WT1. Of note, 2 patients showed increasing levels of WT1 at the planned time-points, as displayed by Figure 1, and they are both in CR at 8 and 12 months from HSCT respectively. The correlation between WT1 kinetics and hematological relapse has been reported as variable after HSCT. In the majority of patients, the elevation of WT1 is quickly followed by relapse (after 30-40 days); in some other patients, the latency from WT1 increase to relapse seems to be longer (Candoni *et al*, Eur J Hematol 2008). The latter occurrence would render the management of immunosuppression potentially more effective. On the other hand our observation might suggest to be cautious about treating patients upon WT1 levels.
Conclusions: Wide studies with long follow-up are needed in order to deepen the kinetics of WT1 post HSCT and thus to hypothesize its prospective clinical use.

**P762**
**Risk-adapted transplant selection improves outcome of adult patients with high-risk AML**
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The outcome of adult AML still remains unsatisfactory due to two main reasons: 1) the risk-category allocation based on the sole definition of pre-treatment biological features may fail to distinguish some high-risk patients, which are not timely addressed to allogeneic SCT (ASCT); 2) the ASCT option may be hampered by the paucity of candidates (25-30%) with a full matched family donor. Nevertheless, upfront genetics/cytogenetics and minimal residual disease (MRD), when properly combined, represent powerful tools to predict relapse risk on an individual basis. We designed a risk-adapted strategy in which high-risk patients, defined according to pretreatment genetics/cytogenetics (poor-risk K or of FLT3-ITD) and/or a positive post-consolidation MRD status (≥3.5x10e-4 residual leukemic cells), should timely receive ASCT whatever the source. For comparison, we analyzed the outcome of a matched historical cohort of high-risk patients who were submitted to ASCT if a full matched family donor was available, or to autologous transplant. The prospective and the retrospective cohort included 23 (4 MRD+ good-K, 9 MRD+ int-K, 4 adverse-K and 6 FLT3-ITD) and 59 patients (8 MRD+ good-K, 33 MRD+ int-K, 1 adverse-K, 10 FLT3-ITD and 7 MRD+), respectively. After a median follow-up of 20 months, survival estimates of the prospective cohort were superior as compared to the retrospective one (DFS 73% *vs* 15%, p=0.011; OS 69% *vs* 20%, p=0.020; CIR 21% *vs* 76%, p<0.001; relapse rate 22% *vs* 69%, p<0.001). All 19 patients in the prospective cohort received ASCT (8 from a matched family donor, 5 haploidentical, 6 matched unrelated donor/umbilical cord blood) as compared to 14/47 (29%) in the retrospective cohort (p<0.001). We conclude that a transplant policy based on a risk-driven "transplant versus no transplant" rather than "donor versus no donor" strategy might improve the outcome of high-risk AML patients.

**P763**
**IKZF1 (IKAROS) deletions as a marker of minimal residual disease in patients with acute leukaemia after alloBMT**
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Objective: The aim of our study was to develop methods for determining minimal residual disease (MRD) for patients with acute leukemia, using deletions in gene IKZF1 (transcription factor IKAROS) as a marker. Methods: The presence of deletions delta 2-7 and delta 4-7 IKZF1 was examined in 95 patients with acute leukemia (AML -26, ALL -64, biphenotypic acute leukemia (BAL) -5) at different stages of disease. Locus-specific primers and TaqMan probes for real-time PCR were designed to quantify deletions delta 2-7 and delta 4-7 mutIKZF1.
Results: The frequency of deletions IKZF1 in the general population of patients with acute leukemia was 11% (n=10), and in patients with ALL, 12% (n=8). But, at the same time, deletions were detected for 31% (5 of 16) of patients with Ph-positive ALL. Two patients with BAL had a deletion in the IKZF1 gene. Within the group of patients with AML and healthy volunteers the above-mentioned mutations were not detected. IKZF1 mutations were found in the group of patients with detected chromosomal aberrations as well as in patients with normal karyotype tumor cells. The IKZF1 gene deletion was not detected in patients at molecular remission. At the same time the number of cells with deletion proportionally increased data on other markers: cytogenetic and molecular (definition of the chimeric transcripts) at relapse. We found out a correlation between mutIKZF1amplification and a number of blasts (R=0.62, p=0.017), chimeric transcripts level (R=0.91, p=0.000001) and donor chimerism (R= -0.63, p=0.02). The sensitivity of mutIKZF1 detection is less then chimeric transcripts level measurement

(88%), but the specificity was observed the same (100%). Conclusion: Deletions in the gene IKZF1 can be used as a marker for evaluation of minimal residual disease in patients with ALL without other informative molecular markers.

**P764**

**The impact of MRD in HCT for childhood ALL**

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Background: Relapse is the major cause of treatment failure after HSCT for childhood ALL. A high level of minimal residual disease (MRD) at HSCT is associated with a high risk of relapse after HSCT.
Materials and Methods: From May 2001 to May 2011, 101 children and young adults with ALL in complete remission (CR) underwent a first allogeneic HSCT at the Clinica Pediatrica in Monza and 82 of them (54 males, median age at HSCT 9,2) could be studied for MRD. 26 patients were in CR1, 46 in CR2, 10 in CR3. HSC source was BM in 67, PB in 11, CB and BM in 3 and CB in 1 and the donor was an HLA identical sibling in 24, an haploidentical parent in 7, and an unrelated donor in 51. Follow-up was up-dated as of November 30, 2011 and the median follow-up was 4 years after HSCT. MRD was analyzed at HSCT and at 1, 3, 6, 9, 12 months after HCT; analyses were performed according to EuroMRD Group guidelines. MRD was considered positive when its level was $5x10^{-4}$ or higher at the time of HSCT and at any level after HSCT.
Results: 54 of 82 patients remained in CCR, 20 relapsed and 8 died of treatment related mortality (TRM). All patients were evaluable for MRD before HSCT and 79%, 83%, 85%, 89% and 77% of the patients at risk could be studied at 1, 3, 6, 9 and 12 months, respectively.
MRD at HSCT. 46 MRD negative patients reported a 5-year event free survival (EFS) of 74.7% (SE 6.6) accounting for 5 relapses and 6 non-leukemic death; 36 MRD positive patients reported a 5-year EFS of 48.8%, (SE 8.9), accounting for 15 relapses and 2 deaths. 5-year EFS in CR1 was 91.7% (SE 8.0) for 12 MRD negative and 55.6% (SE 15.2) for 14 MRD positive patients; 5-year EFS in CR2 was 79.7% (SE 8.1) for 26 MRD negative and 48.5% (SE 11.4) for 20 MRD positive patients. Of 9 patients with MRD $\geq 10^{-3}$, 5 relapsed.
MRD after HSCT. Of the MRD positive patients at 1 (n=19), 3 (n=9), 6 (n=6), 9 (n=11) and 12 months (n=6), 53%, 56%, 100%, 55%, 67% relapsed, respectively. Of the 36 MRD positive patients at HSCT, 22 had at least one positive value after HSCT, which was associated with a subsequent relapse in 13 of the cases, while of the 5 who became persistently negative, 0 developed a relapse. Of the 46 MRD negative patients at HSCT, 36 remained persistently negative, and 2 of them relapsed, and of the 8 patients who became positive at some point after HSCT 3 relapsed.
Conclusions: MRD positivity in transplanted ALL is associated with a dismal prognosis, particularly when $\geq 10^{-3}$ and mostly in CR2 patients.

**P765**

**Match-related allogeneic stem cell transplantation with conditioning included ATG: engraftment kinetics, GvHD, and quantitative chimerism analysis**

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Graft-versus-host disease (GvHD) remains a major complication of allogeneic stem cell transplantation (alloSCT) and has a significant effect on quality of life and mortality. It has been shown that anti-thymocyte globulin (ATG) reduced incidence and severity of acute and chronic GvHD in patients after unrelated alloSCT. A small number of studies suggest the role of ATG in match related (MRD) alloSCT. The aim of this study is to assess the risk of acute and chronic GvHD, survival, mortality, and chimerism date in patients according to receiving ATG prior MRD alloSCT. The prospective single-centre study included 11 patients underwent MRD alloSCT and receiving ATG in additional to conventional GvHD prophylaxis (ATG+). Control group consist 11 MRD alloSCT transplanted patients with cyclosporine/methotrexate prophylaxis (non-ATG). These groups were matched for age, disease, cytogenetic risk group at the time of diagnosis, and disease phase at MRD alloSCT. All patients followed by stable myeloid and platelet engraftment. Leukocyte engraftment in ATG+ and non-ATG groups was at median 16 and 15 days, respectively. Platelet engraftment varied from 12 to 20 days in ATG+ and from 10 to 20 days in control group. On day +28, 91% of ATG+ and 73% of non-ATG patents showed complete chimerism (P 0.03). Chimerism analyses between day +56 and the last follow-up did not reveal any switch of chimerism status. Acute GvHD was evaluable in 55% of ATG+ and in 64% of non-ATG groups. Severe acute GvHD was diagnosed in 18% and 36% patient from ATG+ and non-ATG groups, respectively (P 0.07). Chronic GvHD was evaluable in 67% of patients from ATG+ group and in all patients from control group, who alive more 100 days after alloSCT (P 0.0001). Extended chronic GvHD was present in 22% and 50% of patients from ATG+ and non-ATG groups, respectively (P 0,05). The differences in relapse, mortality, and overall survival between patients received ATG and patients with standard GvHD prophylaxis were not significant.
In conclusion, the addition of ATG to conventional GvHD prophylaxis seems well tolerated, resulted donor engraftment in all evaluable patients of this small cohort. ATG as part of conditioning regimen leads to a significant reduction of GvHD without increase of relapse. Although graft rejection was completely avoided, severe GvHD did occur. ATG may help to decrease the rate or severity of GvHD in this setting.

**P766**

**Semiquantitative column agglutination technology for the confirmation of donor chimerism following allogeneic stem cell transplants**

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Background: Incompatibility in red blood cell (RBC) systems can induce a hemolytic reaction, but not have an impact on outcome following allogeneic stem cell (SC) transplant. Besides clinical, hematological and cytogenetic monitoring, testing for immunohematological markers is useful for confirmation of allogeneic SC engraftment. Outcome of allogeneic SC transplants can be predicted by kinetics of conversion of the blood groups - that is by donor-specific RBC appearance and disappearance of recipient-specific RBC antigens, as well as by verification of specific ABO antibody alterations.
Aim: To investigate conversions of RBC phenotype using semiquantification of the mixed fields in the solid phase (column agglutination technology) after SC transplant, as well as to determine the type and degree of donor himerism. The goal was also to determine the correlation between RBC phenotyping and findings of cytogenetic and molecular testing.
Patients and Methods: Total of 22 patients were underwent to allogeneic SC transplant form HLA-matched, but ABO-incompatible (major, bidirectional or minor incompatibility) donors. In control group, patients by ABO-compatible transplants were treated. Acceptance of the allograft was assessed on the basis of semiquantitative testing of ABO, Rhesus, Kell, Duffy, Kidd, P, MNSs and Lewis blood group antigens before and after transplants - using column agglutination technology in the solid phase (BioVue Ortho System). Analysis of the presence/titer

of ABO antibodies in recipient's serum (ABO-incompatible setting) was also performed.
Results: By RBC phenotyping in solid-phase, the proportion of donor-specific RBCs (DS-RBCs) at levels from 50% to 100% between the 39. and 155. posttransplant day was observed. The existence of 100% of DS-RBCs in the patients' circulation or complete disappearance of recipient-specific RBC antigens on the 87.9±36.2 day after SC transplant was detected in average. Alteration in the specificity and/or titer of ABO alloantibodies was a less important marker of allograft acceptance. A statistically significant correlation between immunohematological findings and results of cytogenetic and molecular testing was confirmed.
Summary/Conclusions: The use of semiquantitative column RBC phenotyping, and investigation of ABO seroconversion considerably could be improve the monitoring of SC engraftment - especially when cytogenetic and molecular testing is difficult or impossible.

**P767**

**Non-malignant host/recipient haematopoiesis following HSCT for leukaemia: lineage specific stable mixed chimerism in relation to clinical course**

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Although mixed Chimerism (CHM) is commonly encountered following HSCT for leukemia, it typically fluctuates. In such patients, the advent of Non-Malignant, Stable Mixed CHM (NM-SMC) is distinctly unusual, and its clinical significance is largely unknown. Since mixed CHM of certain lineages may have negative prognostic implications, three leukemic patients with SMC (based on STRs) were evaluated in relation to PCR-MRD, and lineage specific chimerism for cd3-T, cd19-B, cd56-NK and cd33/66-myeloid cells. Patients were selected that displayed on routine longitudinal, STR-based CHM analysis the following features: (1) SMC (<95% donor) for at least 5 weeks; (2) >6 sequential samples with chimeric variance < ± 5% across samples; (3) PCR initially negative for MRD markers during SMC interval. Duration of NM-SMC and follow-up was: 47/47 wk, 128/128 wk, 43 wk-intermittent/336 wk. Multi-Lineage CHM Analysis (MLA) showed for case #1 (AML) – T cell (mainly) and myeloid MC; case #2 (ALL) – Pan-lineage MC; case #3 (CML) - solitary NK cell MC. Only case #3 with isolated NK cell NM-SMC eventually developed MRD positivity, without clinical relapse. These observations raise points in two directions. In a CLINICAL vein: (1) long-term, SMC may occur that is due to non-malignant recipient hematopoiesis; (2) the risk for molecular relapse may depend on the specific chimeric lineage, e.g. NK cells here. These findings also raise the following TECHNICAL issues in regards to post-transplantation monitoring of leukemia: (1) A longitudinal approach to CHM analysis is necessary to detect the occurrence of SMC; (2) MRD testing is needed to establish that SMC is non-malignant; (3) STR-based CHM analysis, regardless of sensitivity, cannot substitute for specific PCR-MRD evaluation; STR assays do not distinguish malignant from normal, recipient hematopoietic cells; (4) lineage CHM analysis may help identify cases at risk for relapse.
In conclusion, long-term NM-SMC occasionally occurs in leukemic patients after HSCT. Some cases may warrant therapy, despite MRD negativity, due to factors that likely include chimeric lineage. Since the chimeric level is stable in these cases, molecular detection of early relapse must rely on regular evaluation of MRD markers. For these reasons, post-transplantation monitoring should enable detection of stable mixed CHM, its chimeric lineage and MRD status.

**P768**

**Efficacy of a panel of short tandem repeats in chimerism status analysis: informativity of the single markers evaluated on a large number of donor/recipient couple**

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Short Tandem Repeats (STR) polymorphysm analysis is actually the most employed method for the study of Chimerism Status (CS) after Hematopoietic Stem Cell Transplantation (HSCT). Quantitative or semi-quantitative assays based on PCR can do a good assessment of the Donor/Recipient (D/R) ratio in a cellular subset. Despite these considerations, actually there is not a standardized STR panel for the CS study only and many laboratories employ panels used in forensic studies.
Here we present our experience in CS analysis employing a forensic kit (Powerplex 16, Promega USA) in 236 D/R couples: 121 derived from sibling transplants (ST) and 115 derived from unrelated donor transplant (UTD).
Aim of the Study: Primary end point was to evaluate the efficacy of the kit in providing informative markers. Secondary end points was to define the more and the less useful markers in CS analysis considering two different kinds of D/R couples: familiar in ST and not familiar in UTD.
Result: The panel provide at least one informative marker for the CS analysis in 99% of UTD and 98% of SB. The majority of D/R couples had 2 informative markers (42% in ST and 40% in UTD), 19% and 13% had only 1 marker and 39% and 48% had from 3 to 6 markers in ST and UTD respectively. Considering the single markers, we have seen that 10 of them have an high frequency of informativeness either in ST or in UTD, despite there are 3 markers that are useless because rarely significant and never single. The single STR markers in order of significance are: Penta D, TH01, Amelogenin, Penta E, D18S51, TPOX, D13S317, D8S1179, D7S820, D21S11, D16S539, D3S1358, FGA, D5S818, Vwa, CSF1PO.
These data have been confirmed also with a statistical analysis based on the linear regression test which compares the level of significance of each marker in our population with the level of variability of each STR allele in the general population. We have seen a direct correlation of these two variables ($R^2$: 0,20). We have also seen that in our population 12 STR alleles are sufficient to provide at least 1 markers for CS in 99% of D/R couples, but considering the general population and the level of variability of each markers we estimate that only the last 3 ones could be eliminated.
Conclusion: These data confirm the high efficacy of this panel in providing informative markers for CS study. We retain that some STR alleles which have a low variability level in the population could be replaced with other more informative.

**P769**

**Concurrent analysis of chimerism and minimal residual disease of adult T-cell leukaemia/lymphoma with multicolour FACS in a recipient of cord blood transplantation**

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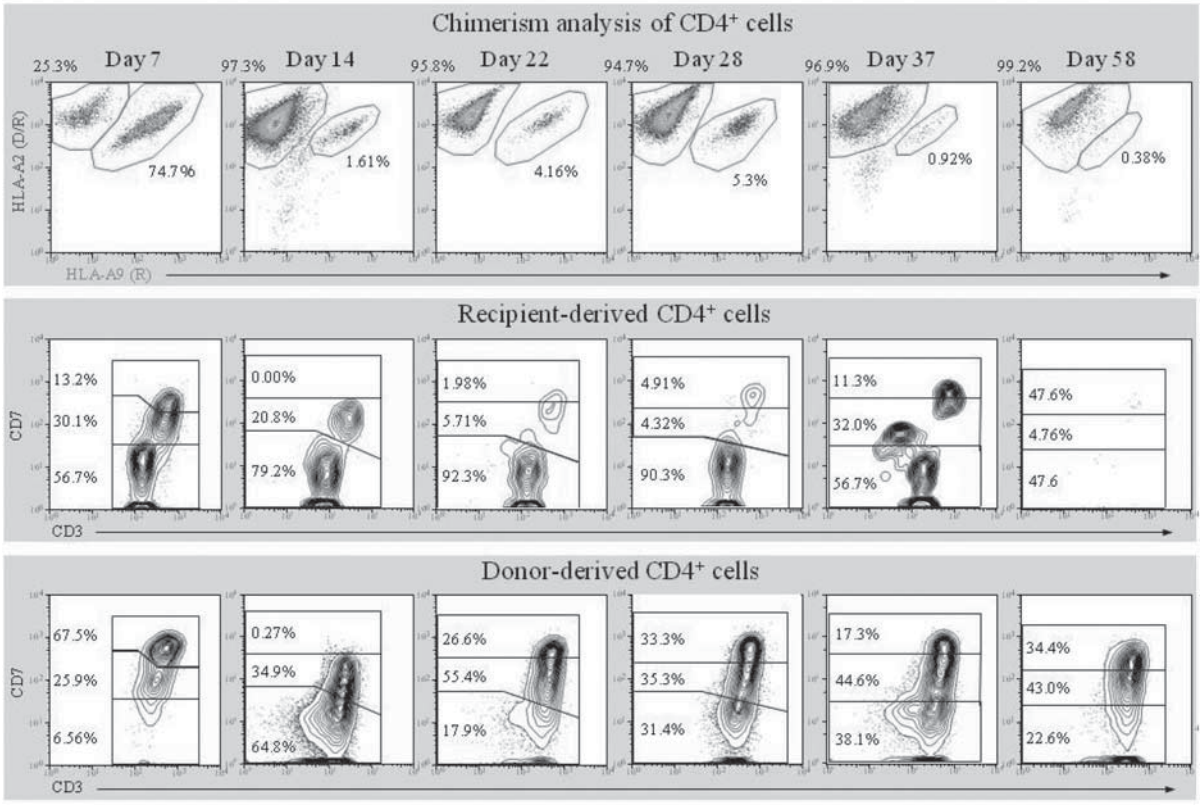
Adult T-cell leukemia/lymphoma (ATLL), human T-lymphotropic virus type 1 (HTLV-1) related hematological malignancy, has been known as one of the chemo refractory diseases. Recently, allogeneic hematopoietic stem cell transplantation (allo-HSCT) has improved overall survival and some reports have suggested that graft versus tumor effect might exist. As in other refractory diseases, umbilical cord blood transplantation (UCBT) has been considered in ATLL patients. Some investigators have sugge-

[P769]

CD4+ T-cell chimerism and MRD of ATLL cell using HLA-Flow method after CBT




sted that a chimaerism study using multicolor FACS analysis with anti-HLA antibodies would be a useful method in HSCT recipients from HLA mismatched donors. On the other hand, using multicolor FACS analysis, a recent study has suggested CD3dim+CD7dim+CD4+ cells and CD3dim+CD7-CD4+ cells are specifically expressed in acute type ATLL. In this study, we report a case which was able to investigate engraftment and minimal residual disease (MRD) simultaneously using multicolor FACS analysis. The patient was a 51 year old female who was diagnosed as acute type ATLL. She underwent UCBT using HLA mismatched cord blood and we analyzed her peripheral blood at 7, 14, 22, 28, 37 and 58 days after SCT. At day 7, 74.7% of CD4+cells were residual recipient type cells. Also, 56.7% of these recipient cells were CD7-cell, so these cells were considered as ATLL cells. At day 14, recipient type CD4+cells rapidly reduced to 1.6%. A brief expansion of recipient's CD4+cells occurred after day 22, however, after day 37 recipient's CD4+cells reduced again. Morphologically, all sorted CD7-CD4+cells of the recipient at day 7 had complicatedly shaped nuclei which were considered as ATLL cells.
In conclusion, as it is a real time study, concurrent analysis of chimaerism and MRD using multicolor FACS will be a clinically useful method for UCBT recipients of ATLL.

**P770**
**Internal validation and quality control in quantitative haematopoietic chimerism testing**
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Quantitative hematopoietic chimerism testing mainly relies on PCR amplification of polymorphic short tandem DNA repeats (STR) and capillary electrophoresis analysis of PCR products. Few studies had addressed STR performance in hematopoietic chimerism testing. Quality control (QC) procedures are routinely used in clinical laboratories. Applications of such procedures to hematopoietic chimerism are scarcely reported. The aim of this study was to asses the impact of STR validation parameters on the quantification of hematopoietic chimerism and to analyze the analytical process through the application of QC procedures.
STR used in this study were SE33, D1S80 and THO1. In addition, PCR for amelogenin and FISH for X and Y chromosomes were used. Analytical chimerism testing was assessed using male/female artificial cellular mixtures prepared in known proportions (0% 0.5% 1% 2% 5% 10% 30% 50% 100%). To evaluate the clinical hematopoietic chimerism performance 152 samples from 96 sex-mismatched transplanted patients were used. The 2% DNA mixture was used to generate Levey-Jennings QC charts. A precision profile for each STR (mixed chimerism range 0.5-30%) was performed. Detection limits for SE33, THO1 and D1S80 were 81, 85 and 61 relative fluorescence units respectively. Analytical sensitivity of artificial DNA mixtures for all STRs was 1% (range 0.5-1.6%). Analytical sensitivity for FISH and amelogenin was 0.5% (range 0.1-1.1%). SE33 and THO1 did not show allelic imbalance while severe allelic imbalance (allele peak ratio <0.60) for D1S80 was detected at 0.25 ng of DNA template mass. The detected allelic imbalance resulted in a 50% overestimation in the mixed chimerism calculation. Sensitivity in clinical samples was 1% (range 0.4-2%) for all STRs. Correlation between the STR and non-STR markers (mixed chimerism range: 0-90%) in clinical samples was high (regression coefficient >0.90). At low mixed chimerism percentage (1%) THO1, SE33 and D1S80 variation coefficient was 23, 19 and 16% respectively.

After performing Levey-Jennings QC charts and applying the Westgard multi-rule procedure violation of the 12SD rule was observed for THO1 and 2 consecutive times for SE33 and D1S80, not complying with the 22SD rule. STR validation is a critical step to detect intrinsic errors that may impact the final mixed chimerism result. Implementing standard QC procedures can identify systematic and random errors so corrective actions can be performed.

**P771**

**Quantitative PCR for Ins/Del polymorphisms is a reliable and sensitive tool for host chimerism analysis after allogeneic hamatopoietic stem cell transplantation**

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The status of Hematopoietic Chimerism (HC) after allogeneic Hematopoietic Stem Cell Transplantation (HSCT) can be an early indicator of adverse events including disease relapse. Classical HC analysis by Short Tandem Repeats (STR) is hampered by a relatively low sensitivity and inter-marker competition in multiplex PCR. In HLA mismatched HSCT, HLA has been shown to be a sensitive marker for HC analysis, however this method does not detect leukemia relapse involving selective loss of the non-shared HLA, a frequent phenomenon in this setting. In order to overcome these problems, in this study we have tested HC analysis by quantitative PCR (Q-PCR) using the AlleleSEQR® Chimerism Research Use Only Assay (Celera Corp., Alameda, CA) which comprises 34 Ins/Del polymorphic markers covering 19 different chromosomes. Using 50ng genomic DNA template, this assay had a sensitivity of 0.2% with excellent inter-marker reproducibility and efficiency (91-100%). HC was studied in 173 follow-up samples of 28 patients after myeloablative haploidentical HSCT for high risk leukemia, comparatively by Q-PCR, STR and HLA. At least 2 different informative markers were found for all patients. The overall concordance of Q-PCR was 71.1% and 72.3% with STR and HLA, respectively. All discordances were due to a positive signal in Q-PCR with a negative result for the other method.
Correlation between HC results and relapse was analyzed in 17 informative patients based on the available time of follow-up. 13 relapses (7 with and 6 without HLA loss) occurred in 11 patients and were predicted in 11/13 (84.6%), 4/13 (30.7%) and 6/13 (46.1%) cases by a positive signal in Q-PCR, STR and HLA, respectively, with a median time from first signal to relapse of 227, 102 and 172 days, respectively. Importantly, 6/7 HLA loss relapses were detected by Q-PCR with a median time from first signal to relapse of 364 days. In 6 patients without relapse during the time of observation, no positive signals were seen by either method with the exception of one patient with a series of positive Q-PCR results during active acute graft versus host disease which turned negative after successful treatment of the condition. Overall, the positive and negative predictive value was 92% and 71% for Q-PCR, 100% and 40% for STR and 100% and 46% for HLA.
Taken together, our data show that Q-PCR for Ins/Del polymorphisms holds promise for improving the sensitivity and informative value of HC in allogeneic HSCT.

**P772**

**Acute GvHD is a strong predictor of full donor CD3+ T-cells chimerism after reduced-intensity conditioning allogeneic stem cell transplantation**

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The monitoring of chimerism is a standard procedure to assess engraftment and achievement of full donor lymphoid cells after RIC. However there is no consensus on when and how often to perform post-transplant chimerism.
We retrospectively analysed our experience about the impact of acute GvHD in the prediction of allograft chimerism. All patients with hematologic malignancies, transplanted between 2001 and 2010 after Fludarabine-Busulfan-ATG RIC from a HLA identical donor and with T cells chimérisme (TCC) determination between day 30 and 120 were included. 115 patients fulfilled all criteria. Allo-SCT was performed from familial donor in 92 patients (80%) and from MUD in 23 patients (20%). The conditioning regimen consisted of fludarabine 90 to 180 mg/m², Busulfan 8 mg/kg orally or 6.4 mg/kg iv and ATG 2.5 or 5 mg/kg. TCC was serially assessed at 30, 60 and 90 days after allo-SCT. Recipient peripheral blood T lymphocytes were positively sorted by a mix of anti-CD4 and CD8 immuno-magnetic beads (Dynal, Compiègne, France). T-cell purity was controlled by flow cytometry and was always ≥95%. Genomic DNA was amplified using fluorescent PCR primers for polymorphic variable number tandem repeats (VNTR) or short tandem repeats (STR). Mixed T-cell chimerism was defined as between 5 and 94% recipient cells, and full chimerism was defined as the presence of more than 95% donor cells.
Full TCC was achieved in 94 patients (82%) at a median of 77 (30-120) days post transplant. Fifty eight patients (50.4%) developed acute GvHD. The cumulative incidence of Grade 2-4 GvHD in our population is 32% (95% CI 23-41).
Overall the results showed that each of the 37 patients developing grade ≥2 AGVHD had a Full TCC prior day 120. On the other hand, all mixed chimerism were documented in patients not presenting Grade≥2 AGVHD (21 of the 78 patients (27%) without grade ≥ 2 AGVHD) (p=.002). No other parameter (ATG dose, Donor type…) achieved this level of individual prognostic.
These results, in a very homogenous population, raise the question concerning the utility of routine chimerism surveillance in patients presenting an acute GvHD following RIC Allo-SCT and that can imply a not negligible saving in terms of economic and human resources.

**P773**

**Early peripheral blood and T-cell chimerism dynamics after single cord blood transplantation with co-infusion of CD34+ cells from a third party HLA-mismatched donor (dual transplant) predicts cord blood graft failure**

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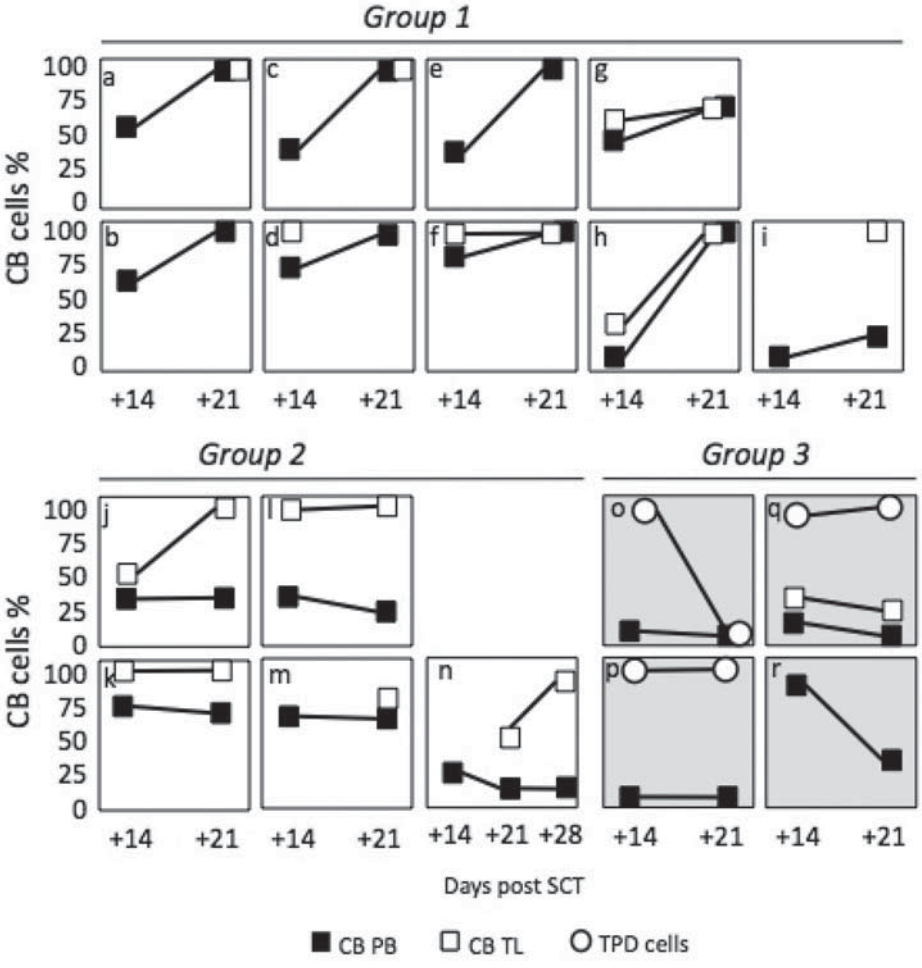
Umbilical cord blood (CB) transplant is an alternative for patients with hematological malignancies lacking HLA-matched adult donors for allogeneic transplantation. The co-infusion of CD34+ cells from a third party HLA-mismatched donor (TPD), so called dual transplant, has shown to reduce the period of postransplant neutropenia and related complications of single CB transplant. The aim of this study was to analyze the value of early postransplant peripheral blood (PB) and T lymphocytes (TL) chimerism analysis after dual transplant regarding CB engraftment.
Methods: 17 patients underwent 18 dual transplants between 2004 and 2011. Chimerism analysis was performerd weekly until complete chimerism (CC) by STR-PCR (AmpFlSTR SGM Plus; Applied Biosystems) in PB and TL. CC was defined as

<1% recipient in PB and <5% in TL (95% purity of enriched samples).
Results: From the 18 transplants, 12 (Figure 1a-n) showed engraftment (>500 TNC) in a median of 16 days (11-48) reaching full CB chimerism in a median of 24 days. Early postransplant PB chimerism showed increasing CB cells in 9 cases (Figure 1a-i). Only 2 cases showed low (<15%) percentages of CB cells on day +14 (Figure 1h-i), however both exhibited a significant increase in the second sample (day +21). In the remaining 5 cases the proportion of CB cells in PB remained stable or decreased from day +14 to day +21 (Figure 1j-n), however TL chimerism showed a significant increase or remained near 100% CB cells in both samples. One case showed low percentages of CB on days +14, +21 and +28 (Figure 1n) together with TNC<500 compatible with graft failure, however CB cells in TL showed a significant increase on day +28. This patient subsequently engrafted obtaining full CB chimerism. On the other hand, 4/18 transplants experienced CB graft failure

**[P773]**

**Table 1. Patient and Transplant Characteristics**

| | |
|---|---|
| **N** | 18 (17 patients) |
| **Gender** | 9F / 8M |
| **Age** | 33 (22-58) |
| **Diagnoses** | 8 AML; 1 BC-CML; 5 ALL<br>1 DLCBL; 1 HL; 1 MDS |
| **Conditioning** | FLU-BU-Cy-ATG (n=16)<br>TT-FLU-ATG (n=1)<br>TBI-FLU-Cy-ATG (n=1) |
| **GvHD prophylaxis** | CyA + steroids (n=17)<br>CyA (n=1) |



(Figure 1o-r) showing low percentages of CB cells in PB on day +14 with a decrease on day +21 (Figure 1o-q) or an initial high proportion with a significant decrease in the following sample (Figure 1r).
Conclusions: Early postransplant chimerism dynamics in PB and TL predicts CB engraftment or failure in dual transplants. Initial low percentages of CB cells in PB (<15%) without an increase within the first month postransplant as well as a decrease in the proportion of PB CB cells without an increase in TL, seem to correlate with CB failure. Therefore, an early significant proportion of CB in TL associates with CB engraftment irrespectively of the dynamics of chimerism in PB.

**P774**

**Long-term persistence of triple-donor mixed chimerism after double unrelated cord blood rescue transplantation**

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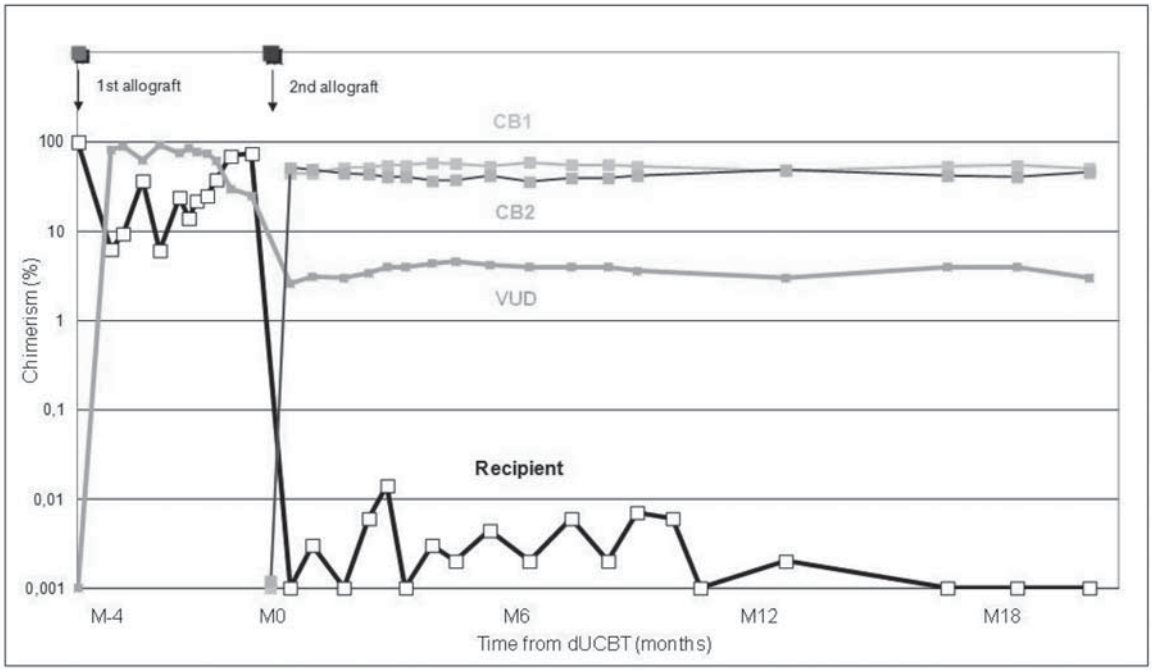
Background: Double unrelated cord blood transplantation (dUCBT) is increasingly used in adults. After dUCBT, one cord blood (CB) unit usually emerges as the dominant one and is responsible for long-term immune reconstitution. However, long-term mixed chimerism has also been described. We report here the case of a patient who received a dUCBT as rescue after hematopoietic stem cell transplant (HCT) from a voluntary unrelated donor (VUD) and whose long-term chimerism showed the persistence of cells from the 3 grafts.
Case Report: A 50-year-old man was diagnosed with stage IV diffuse large B-cell lymphoma and received 8 cycles of R-CHOP leading to complete remission (CR). He relapsed 7 months later and achieved CR2 after 4 cycles of R-ICE. After consolidation chemotherapy, he received a 9/10 HLA-matched VUD HCT conditioned with 4-Gy total body irradiation, cyclophosphamide and antithymocyte globulin (ATG). Chimerism analysis was done by either real time PCR of In/Del polymorphisms for the minority component or STR PCR, on total nucleated cells (TNC) of peripheral blood (PB). At 7 weeks, a mixed chimerism with 37% recipient cells was documented, leading to stop cyclosporin. After transient improvement of chimerism, pancytopenia occurred and graft rejection was confirmed by (i) a marrow biopsy showing severe hypoplasia and (ii) 75% recipient cells in PB. A rescue dUCBT was urgently performed after conditioning with fludarabine, cyclo-

**[P774]**

| | TNC (%) | CD3+ cells (%) | CD19+ cells (%) | CD56+ cells (%) |
|---|---|---|---|---|
| VUD | 4 | 5 | 4 | 4 |
| CB1 | 55 | 65 | 53 | 49 |
| CB2 | 41 | 30 | 43 | 47 |

**Table 1: Chimerism on TNC and on CD3+ T-cells, CD19+ B-cells and CD56+ NK-cells, 7 months after dUCBT.**

**Figure 1: evolution of chimerism over time.**



phosphamide and ATG. Both CB units (CB1 and CB2) were matched 4/6 with the recipient and the VUD, and 5/6 with each other. Two weeks after dUCBT, chimerism showed 45% cells from CB1, 52% from CB2 and 2.6% cells from the VUD. This feature, with an unexpected long-term detection of the VUD cells (3 to 4.6% of TNC) and of the 2 CBs in similar proportions, persisted over time (Figure 1). Seven months after dUCBT, chimerism was performed on the CD3+, CD19+ and CD 56+ cell compartments and showed results comparable to TNC (Table 1). At 21 months after dUCBT, this uncommon triple-donor immune reconstitution is still observed and stable, and associated with polyclonal hypergammaglobulinemia around 40 g/L.
Conclusion: This case confirms the rare occurrence of long-term mixed chimerism after dUCBT, but additionally suggests (i) that mixed chimerism may be equitable between 2 CB units, (ii) that it may concern several cell lineages and (iii) that dUCBT could be associated with a tolerance status allowing long-term detection of VUD cells.

**P775**

**Achievment of full donor chimerism and minimal residual disease clearance with early dasatinib therapy after intra-bone cord blood transplantation for Philadelphia positive acute lymphoblastic leukaemia: a case report**

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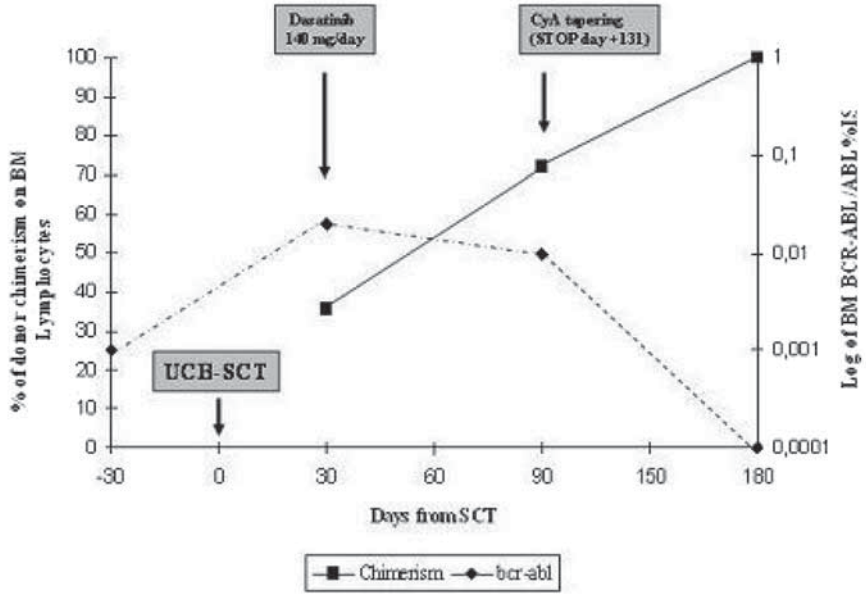
Objectives: The prognosis of Philadelphia positive Acute Lymphoblastic Leukemia (Ph-ALL) is very dismal, with almost 60% of patients relapsing after allogeneic stem cell transplantation (allo-SCT). Early therapy with tyrosine-kinase inhibitors (TKIs) (imatinib, nilotinib and dasatinib) has changed the therapeutic approach with the aim to reduce the relapse risk.
Methods: We report the case of a 43 years old woman with Ph-ALL and molecular biology positive for e1a2 BCR-ABL fusion gene, encoding for P190 tyrosine kinase protein.
The patient was enrolled in a pilot study and was transplanted on 20th April 2011 with a HLA antigen 6/6 matched cord blood (CB) unit. The total nucleated cell count before thawing was 2.07 x $10^7$/kg (CD34+ count of 1.4 x $10^5$/Kg. The patient was conditioned with Thiotepa 10 mg/kg, Fludarabine 125 mg/m$^2$, Ciclofosfamide 100 mg/kg and Thymoglobuline 5 mg/kg. Graft versus host disease (GVHD) prophylaxis consisted in cyclosporine 1 mg/kg from day -7 to day +120 and mycophenolate mofetil 15 mg/Kg bid from day +1 to day +27. G-CSF was administered from day +2 until neutrophil recovery on day +14 (ANC>500/mm$^3$). Platelet recovery (PLT>20x$10^9$/L) was observed on day +20. No major complications developed during the aplastic phase. The molecular monitoring of minimal residual disease and donor chimerism on bone marrow is reported in Figure 1. On day +30 donor chimerism was 35.8% and residual disease was 0.02 BCR-ABL/ABL% IS, so we started dasatinib therapy (140 mg/day) and reduced cyclosporine that was definitively withdrawn on day +131. Any toxicities were observed during dasatinib therapy that is still ongoing.
Results: Pre-emptive dasatinib treatment determined a progressive decrease in molecular transcript levels and an improvement in donor chimerism (Figure 1). At last follow up, 6 months after transplant, the bone marrow chimerism is 95.4% donor on lymphocytes and 96.7% donor on neutrophils and the minimal residual disease is undetectable at molecular level. No GVHD was developed.
Conclusion: The competition of donor stem cells and leukemic stem cells is probably a key point for success of the transplan-

**[P775]**

**Figure 1.**

**Bone Marrow molecular monitoring of minimal residual disease and donor chimerism of a patient with Ph-ALL treated with early dasatinib administration after UCB transplantation.**



tation and an early use of TKIs may give a significant contribution in disease eradication with a possible synergism of immunosuppression withdrawal and dasatinib therapy. A longer observation time is needed before drawing further conclusions and prospective trials are necessary to address some questions about choice of TKI therapy after allo-SCT.

**P776**

**Donor-recipient chimerism analysis of CD3(-) cells is a useful tool for prediction and early detection of relapse after allogeneic stem cell transplantation**

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Donor-Recipient Chimerism (D/R chimerism) analysis of CD3(+) cells is recognized as an important diagnostic tool in allogeneic stem cell transplantation (ASCT) . In this study, we evaluated the usefulness of D/R chimerism of CD3(-) cells as a prediction marker and an early detection marker after ASCT.
Methods: Fifty hematological malignancy patients receiving ASCT were enrolled in this study. We obtained 30 ml of peripheral blood samples on days 30, 60, 120, 150, 180 after a written informed consent. Mononuclear cells (PBMC) were collected using Lymphosepal separation solution and CD3(+) cells were separated using the MACS system according to the manufacture's protocols. After obtaining DNA from PBMC, CD3(+) cells, CD3(-) cells and granulocyte, STR analysis were proceeded using AmpFLSTR® Identifiler PCR Amplification Kit. We also tested other molecular markers including WT1 mRNA quantitative analysis, and evaluated the relationship between CD3(-) chimerism and clinical diagnosis.
Result and Discussion: Fifty patients (AML 24pts, MDS overt AML 5pts, MDS 9pts, CMMoL 1pt, and ALL 11pts) receiving ASCT were enrolled in this study. Thirteen pts. relapsed, 8 pts. died without relapse and 1 pt. withdrew the consent until day 180. Median relapse date is day 89 (32-164) and MST after AST is day 255. Thirteen patients of 50 pts. showed ≥2 percent decrease of CD3(-) chimerism, and 10 pts. from the 13 pts. showed early relapse and one showed non-relapse death. Two pts. finally reached 100% chimerism of CD3(-) cells and still alive. All of 10 hematological relapse patients showed decrease of CD3(-) chimerism without decrease of CD3(+) chimerism nor granulocyte chimerism.
As results of our experiments, 80% of patients showing ≥ 2% decrease of CD3(-) chimerism relapsed until day 180 after ASCT, especially 90 % of pts. with ≥ 2% decrease of CD3(-) chimerism without decrease of CD3(+) chimerism relapsed. These results suggested mixed chimerism of CD3(-) cells is a prediction marker of relapse and ≥ 2% decrease of CD3 (-) cell chimerism is an early detection marker of relapse.

**[P776]**

**D/R chimerism of CD3(-) cells vs. relapse**

| N=39 | Chimerism >2%↓(+) | Chimerism >2%↓(-) |
|---|---|---|
| Relapse death | 10 | 0 |
| Complete remission | 2 | 20 |
| Non-relapse death | 1 | 6 |

**P777**

**A case of long-time persistence of 2 units of cord blood in patient with SCID**

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The effectiveness of the cord blood units (CBU) hematopoietic stem cell transplantation (HSCT) in severe combined immune deficiency (SCID) is undoubted. Usage of 2 units shows the advantages in cellularity and fast engraftment. Also, the engrafted unit has the highest rate of T-cells. The long-time persistence of 2 CBU in linear chimerism is very rare situation.
Case Report: The reported case is 1 y.o. girl with SCID (T-B-NK+) complicated by enterocolitis, hepatosplenomegaly, lymphadenopathy, symptoms of hemophagocytosis and generalized BCG infection. Gancyclovir therapy was initiated according to the CMV-viremia persistence. Conditioning regimen: Fludarabine 150 mg/sq.m., Melphalan 140 mg/sq.m. and Timoglobulin 10 mg/kg. HSCT of 2 CBU (9/10 HLA-DRB1 mismatch) was realized on day 0. Total NC dose – $5x10^7$/kg, CD 34+ $10^6$/kg. Cord blood units were HLA-identical but with different Rhesus factor. GvHD prevention: MMF, tacrolimus. Engraftment was registered on day +13. Skin GvHD onset was registered on day +28 and showed the undulating course till the present time. Now on day +380 the limited cGvHD is observed. GvHD treatment: corticosteroids, cyclophosphomide and etanercept. On day +60 the Rh-conflict developed with expressed clinical picture during 1 week. Immunoreconstitution was registered on day +200. Fisher-Evans syndrome (FES) developed on day

**[P777]**

| | *Day +30* | | *Day +120* | | *Day +270* | | *Day +360* | |
|---|---|---|---|---|---|---|---|---|
| | CBU 1 (%) | CBU2 (%) | CBU 1 (%) | CBU2 (%) | CBU 1 (%) | CBU2 (%) | CBU 1 (%) | CBU2 (%) |
| **Chimerism** | **40** | **60** | **0** | **98** | **16** | **84** | **0** | **98** |
| CD19 | 42 | 58 | | | | | | |
| CD56 | 53 | 47 | | | | | | |
| CD3 | 32 | 68 | | | 9 | 91 | | |
| CD14 | 31 | 68 | | | | | | |
| CD15 | 90 | 10 | | | 37 | 63 | | |

+210 (treatment – 4 infusions of rituximab). Prolonged chimerism research showed the persistence of 2 CBU during 1 year in different percentage (Table 1).
Discussion: The described material shows the unique case of long-time-persistence of 2 CBU that leads to the serious alloimmune reactions: FES, Rh-conflict etc. This situation requires the additional immunosuppressive therapy, special warnings on the chimerism monitoring and blood serology. Intensive immunosupression and HLA-identity lead to the simultaneous existence of 2 CBU. But the significantly fast engraftment achieved in case of such type HSCT.
Conclusion: HSCT of 2 CBU shows the several advantages in case of SCID. The special attention must be paid on GvHD prevention and treatment. The described material shows the importance of the linear chimerism investigation as a control method. The usage of this type HSCT (in case of possibility) is indicated for SCID patients in such severe condition. The important issue that the usage of HLA-identical between each over CBU is not recommended.

**P778**

**Relapse after allogeneic haematopoietic stem cell transplantation in haematological malignancies: factors impacting its occurrence and treatment options for a better management**

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To evaluate the different pre- and post-transplantation factors impacting the relapse occurrence after allo-HSCT, and to evaluate factors impacting the survival post-relapse including the different treatment options; we retrospectively studied the occurrence of relapse in 345 patients, 198 (57%) males and 147 (43%) females with a median age of 43 years (range 17-66) who received allo-HSCT at our institution for hematological malignancies between years 2000 and 2011; 205 (59%) from siblings donors and 140 (41%) form unrelated donors. At transplantation, there were 148 (43%) patients in first complete response or first chronic phase (CR1/CP1), 66 (19%) in CR2/CP2 and 131 (38%) < CR2/CP2; 206 (60%) patients received a full intensity conditioning and 139 (40%) a reduced intensity one. The different patients and transplantations characteristics are described in the Table 1. After HSCT, 336 (97%) patients engrafted. The cumulative incidence of acute GVHD≥2 at 3 months was 35% (95%CI 32-37); the cumulative incidence of extensive and limited chronic GVHD at one year was the same 15% (95%CI 13-17). After a median follow-up of 11.4 months (range 4-129), the median overall survival (OS) for the whole population was 19 months (range 12-33) with a 2-years probability of 47% (95%CI 42-53). Eighty eight (25.5%) patients relapsed with a cumulative incidence at one and two years of 19% (95%CI 17-21) and 22% (95%CI 20-24) respectively. After relapse, 65 (74%) patients were treated [21 (32%) received DLI alone, 21 (32%) chemotherapy alone, 14 (22%) DLI + chemotherapy and 9 (14%) received other treatment] and 23 (26%) were not treated due to deadly relapse. The median OS from relapse was 4 months (range 3-5) and the one year probability of OS in patients who relapsed was 21% (95%CI 14-31). The multivariate analysis studying the impact of different variables on the occurrence of relapse showed a negative impact of disease status [<CR/CP: HR=3.9 (2.4-6.7), p=0.0001], a negative impact of CMV status [D+R-: HR=2.4 [1.2-4.7], p=0.009] and a protective impact of cGVHD [HR=0.37 (0.2-0.6), p=0.0002]. The multivariate analysis studying the pre- and post-relapse variables on the survival after relapse showed a positive impact of Major ABO incompatibility: HR=0.39 (0.16-0.9), p=0.03], a negative impact of disease status [<CR/CP: HR=2.4 (1.3-4.4), p=0.003] and a positive survival outcome in patients receiving DLI with or without chemotherapy [HR=0.5 (0.3-0.8), p=0.005].

**P779**

**Assessment of efficacy (or lack thereof) of donor lymphocyte infusion in relapsed lymphoid malignancies following haematopoietic cell allografting: results of a systematic review**

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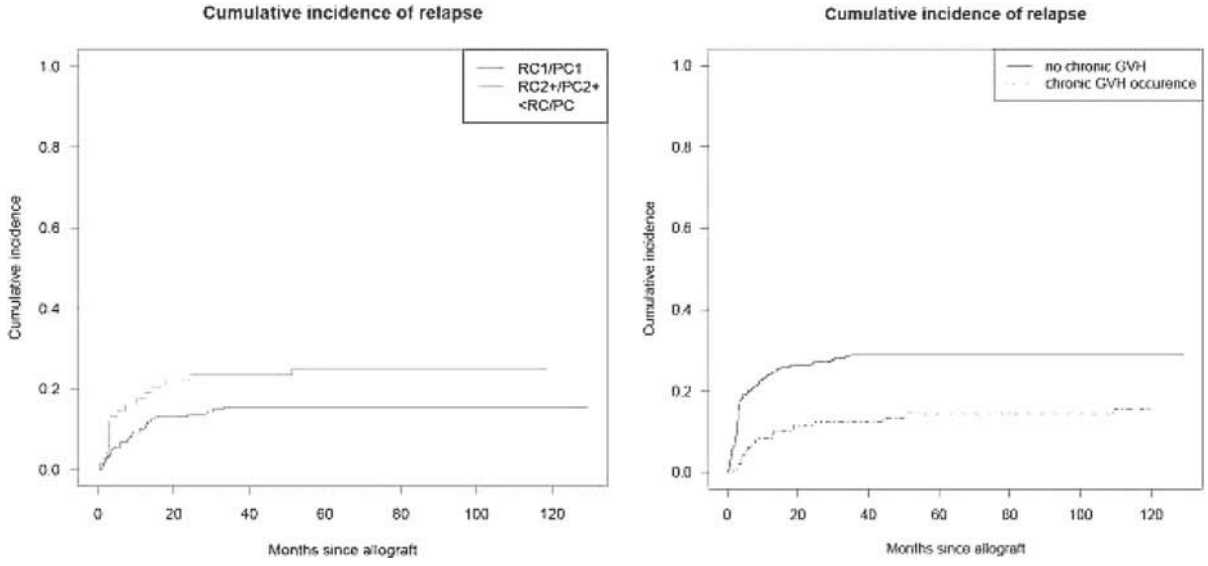
Background: Adoptive immunotherapy through the bona fide graft-versus-malignancy (GVM) effect, mediated by DLI, can eradicate disease relapse after allogeneic hematopoietic cell transplantation (allo-HCT). Efficacy of DLI is more marked in CML versus other myeloid neoplasms such as AML or MDS. Benefit (or lack thereof) of DLI when lymphoid malignancies relapse after allo-HCT is less known; and not predictable across various subtypes.
Methods: We searched published literature using a broad strategy to identify prospective or retrospective studies evaluating DLI in relapsed lymphoid malignancies (acute lymphoblastic leukemia (ALL), chronic lymphoblastic leukemia (CLL), multiple myeloma (MM), and lymphomas (Hodgkin (HL) or non-Hodgkin (NHL)) between 1966 and 06/30/2011. We identified 403 (PUBMED= 392,

**[P778]**



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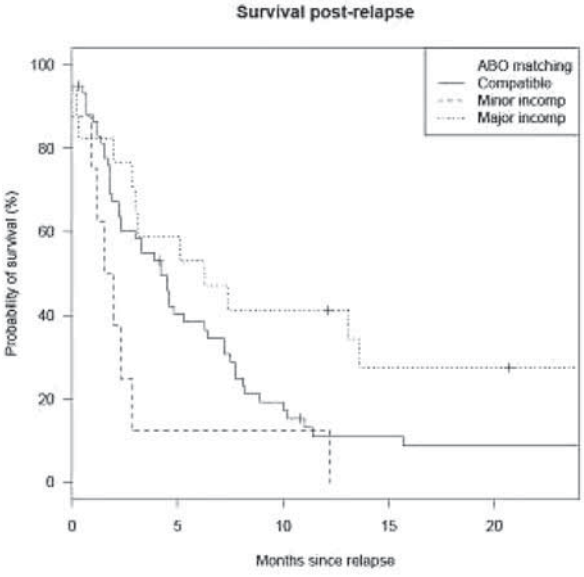


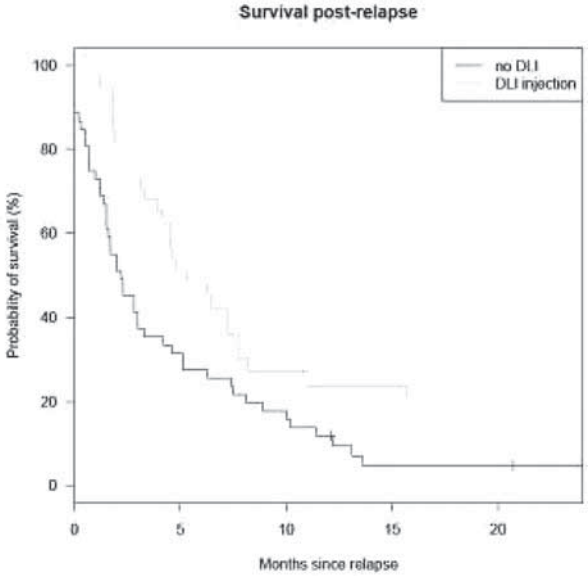


| | Main population N=345 | Relapse population N=88 |
|---|---|---|
| **Gender** | | |
| Male / Female | 198 (57%) / 147 (43%) | 45 (51%) / 43 (49%) |
| **Age** | | |
| Median, years | 43 (17-66) | 46 (19-63) |
| **Disease** | | |
| Myeloid leukemia | 228 (66%) | 65 (74%) |
| AML / MDS | 147 / 32 | 47 / 9 |
| CML / MPS | 31 / 18 | 7 / 2 |
| Lymphoid leukemia | 228 (34%) | 23 (26%) |
| ALL / CLL / Lymphoma | 82 / 16 / 19 | 20 / 2 / 1 |
| **Disease status at HSCT** | | |
| CR1/CP1 | 148 (43%) | 23 (26%) |
| CR2/CP2 | 66 (19%) | 17 (19%) |
| <CR2/CP2 | 131 (38%) | 48 (55%) |
| **Conditioning** | | |
| Full / reduced intensity | 206 (60%) / 139 (40%) | 42 (48%) / 46 (52%) |
| **Cell source** | | |
| PBSC / BM / CB | 123 (36%)/ 194 (56%) / 28 (8%) | 43 (49%) / 40 (45%) / 5 (6%) |
| **Sex-mismatching** | | |
| Fd-Mr / Md-Fr | 84 (24%) / 83 (24%) | 19 (21%) / 25 (28%) |
| **CMV-mismatching** | | |
| D- R+ / D+ R- | 73 (21%) / 49 (14%) | 21 (24%) / 17 (19%) |
| **ABO-mactching** | | |
| major / minor incomp. | 80 (23%) / 52 (15%) | 17 (19%) / 8 (9%) |
| **Donor** | | |
| Siblings / Unrelated | 205 (59%) / 140 (41%) | 53 (60%) / 35 (40%) |
| **Interval diag-HSCT** | 9 months (0.2-219) | -- |
| **Treatment post-relapse** | | |
| DLI | -- | 21 (24%) |
| DLI + chemotherapy | -- | 14 (16%) |
| Chemotherapy alone | -- | 21 (24%) |
| Other | -- | 9 (10%) |
| No treatment | -- | 23 (26%) |
| **RESULTS** | | |
| **Egraftment** | 120 (99%) | 60 (95%) |
| **CI aGVHD≥2 at 3m.** | 35% (32-37) | 27% (23-32) |
| **CI cGVHD at 12m.** | | |
| Limited & Extensive | 15% (13-17) | 8% (8-11) 14% (10-17) |
| **Median OS** | 60 months (31-NR) | 18 months (11-NR) |
| **CI relapse at 12m.** | 17% (14-20) | 15% (9-20) |
| **CI TRM at 12m.** | 12% (9-15) | 33% (27-39) |

manual search=11) studies, but only 39 (single-center=19, multicenter/registry=20) met eligibility criteria (≥4 evaluable subjects (and data extractable) and published in English). Results: pooled-proportion of complete (CR) and overall response (ORR) rates as well as incidence of acute (a), chronic (c), or acute/chronic (a/c) GVHD were: ALL (12 manuscripts) [CR=0.27 (95% CI=0.16-0.20), ORR=0.328 (95%CI=0.206-0.462), aGVHD=0.50 (95% CI=0.39-0.62), cGVHD=0.26 (95% CI=0.17-0.37) and a/cGVHD=0.49 (95%CI=0.30-0.68)], CLL (4 manuscripts) [CR=0.55 (95% CI=0.15-0.92), ORR=0.616 (95% CI=0.306-0.881), a/cGVHD=0.77 (95% CI=0.31-1.00)], MM (13 manuscripts) [CR=0.257 (95% CI=0.190-0.330), ORR=0.51 (95% CI=0.43-0.59), aGVHD=0.53 (95% CI=0.44-0.63), cGVHD=0.375 (95% CI=0.301-0.453), a/cGVHD=0.52 (95% CI=0.38-0.65)], HL (7 manuscripts) [CR=0.367 (95% CI=0.197-0.556), ORR=0.582 (95% CI=0.448-0.710), aGVHD=0.42 (95% CI=0.27-0.59), a/cGVHD=0.578 (95% CI=0.381-0.763)], and lymphomas (HL and NHL) (15 manuscripts) [CR=0.449 (95% CI=0.327-0.574), ORR=0.559 (95% CI=0.443-0.671), aGVHD=0.406 (95% CI=0.311-0.505), cGVHD=0.45 (95% CI=0.34-0.56), a/cGVHD=0.594 (95% CI=0.500-0.684)]. Survival outcomes could not be pooled due to diversity in reporting this outcome. Limitations: a. lack of uniformity in response criteria used amongst studies within the same disease entity, b. criteria to assess GVHD was not uniform and was undefined in some cases, and c. dose, timing, frequency and use of cytoreductive therapy pre-DLI was not uniform.
Conclusion: Responses to DLI appear higher in CLL and lymphomas, and less pronounced in ALL and MM; but findings have obvious limitations of lack of comparison and observational data.

**P780**

**Recovery of KIR expression after allogeneic stem cell transplantation in multiple myeloma patients**

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Introduction: Activating and inhibitory killer immunoglobulin like receptors (KIR) are predominantly expressed on natural killer (NK) cells. KIR mismatch allogeneic stem cell transplantation (alloSCT) has been reported to provide beneficial effects for Multiple Myeloma (MM). However, their recovery in MM patients remains poorly understood. We, therefore, analysed KIR recovery in 90 MM patients after alloSCT.
Methods: KIR expression (CD158a/h, CD158b/b2, CD158e1/e2) on NK cells and T cell subsets was measured by flow cytometry at different time points after alloSCT.
Results: During the first 90 days after alloSCT NK cells represent the largest lymphocyte subset. Activating receptors like NKp30 and NKp44 showed a fluctuating expression while members of the KIR family were expressed at a constant rate (20% of NK cells). There was no significant difference in the early post transplantation period (day 0-90) compared to later time points (day 360).
In contrast, T cells showed increased KIR expression during the first 30 days after alloSCT, which was highly significant for CD158e (p=0,0001). After 30 days the expression declined to baseline. Furthermore, T cell activation marker HLA-DR reached its highest expression between days 60 and 90 when KIR receptors were expressed at their lowest level (27% *vs.* 8%, p<0,0001).
Conclusions: We conclude that KIR receptors were differentially expressed on NK and T cells. Because KIR receptors are constantly expressed by NK cells and NK cells are the most frequent lymphocyte populations early after alloSCT, NK cells may be useful for KIR mismatch cellular therapy.

**P781**

**Immune reconstitution following CD34 selected grafts and DLI from HLA match-related donors in childhood acute leukaemia**

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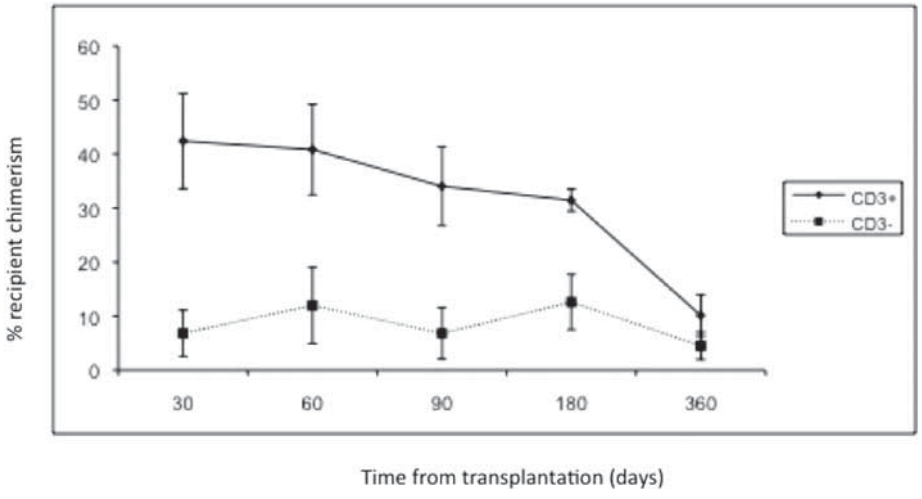
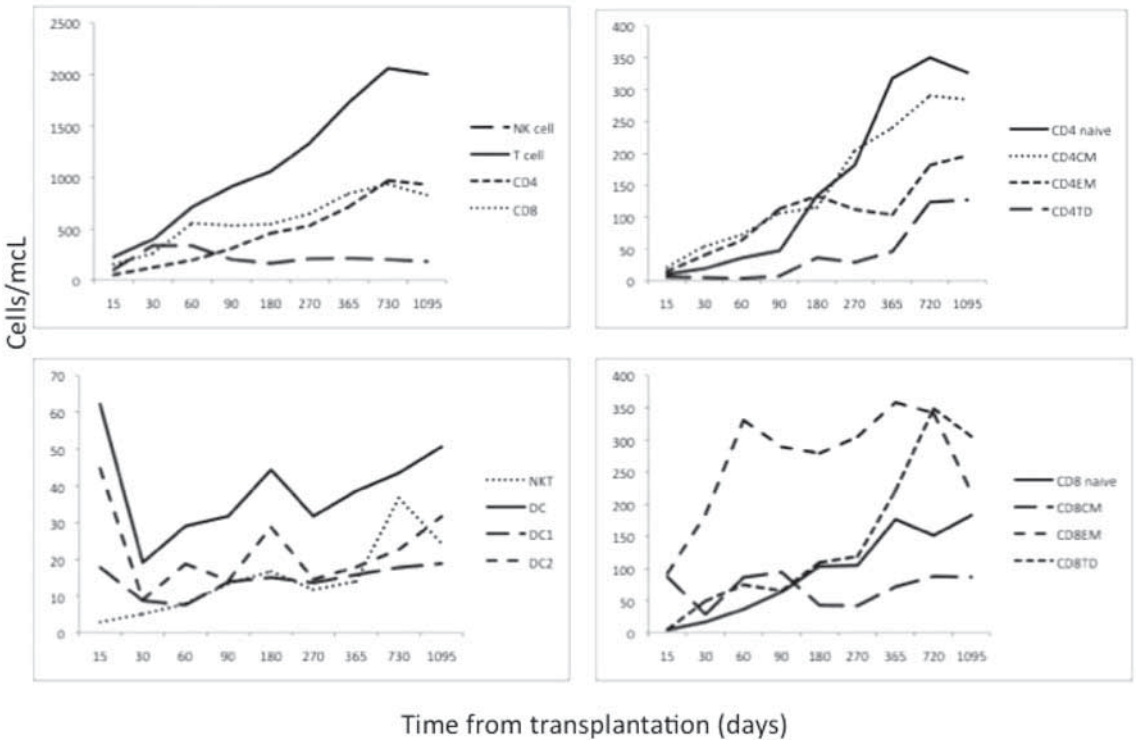
Introduction: CD34+ selected HLA match related donor peripheral blood progenitor cell (PBPC) transplantation followed by donor lymphocyte infusion (DLI), as adoptive immunotherapy, constitutes a new approach to enhance graft-versus-leukaemia (GvL) effects with no significant graft-versus host disease (GvHD). Donor lymphocyte infusion promotes a fast immune reconstitution.
Objectives: We prospectively investigated immune reconstitution and CD3+/CD3- chimerism in 43 CD34+ selected HLA match related transplantations followed by DLI for high-risk childhood leukaemia using a fludarabine-based reduced intensity-conditioning regimen. Graft-versus-host disease prophylaxis consisted of cyclosporine ± methotrexate. Graft consisted of a median of $4.87x10^6$/kg CD34+ and $3x10^5$/kg CD3+ cells. A median of 2 DLI (range 0-8) with 1x106 CD3+/Kg were infused after transplant.
Results: After transplantation 42/43 patients engrafted. The level of CD3+ cell donor chimerism was progressively increasing after transplantation (Figure 1). T cell led lymphocyte immune recovery. T cell subset was inverted until two years after transplantation. Natural Killer cells and CD8 T cells achieved values above 250 cells/mcl

**[P781]** Figure 1



[P781]

Figure 2




in the first 30 days after transplantation. CD4CM and CD8EM T cells rapidly increased after early transplantation. Dendritic cell (DC) recovery was dominated by the DC2- subset (Figure 2). With a median follow-up of 3 years, disease-free-survival (DFS) was 73±7%. Acute and chronic GvHD incidence was 17% and 35%, respectively. Upon univariate analysis high CD3 negative donor cell chimerism, high DC2 and CD8TD effectors T cell population on day 60 were significantly correlated with disease free survival.
Conclusion: DLI after CD34 selected HLA match related donor PBPC transplantation resulted in fast immunological recovery avoiding severe GvHD and preserving the GvL effect mediated by T cell and DC interaction in a mixed chimerism setting.

**P782**
**Lower TGFß serum levels and higher frequency of IFNgamma-producing cells during early immune reconstitution in surviving children after allogeneic stem cell transplantation**
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Allogeneic hematopoietic stem cell transplantation (SCT) is increasingly used as a salvage therapy for patients with high-risk malignancies as well as life-threatening nonmalignant diseases. However, only limited data about the association between outcome and functional parameters of recovering lymphocytes are available so far. In this prospective study of 20 pediatric SCT recipients, we serially evaluated immune parameters quantitatively and qualitatively before and throughout allogeneic SCT. This data was analyzed with respect to survival. Age, gender, GvHD, and type of graft were not different between relapsing and nonrelapsing patients. Notably, in our cohort there was no case of transplant-related or infectious mortality. However, with the exception of two patients with advanced MDS, all patients not in complete remission (CR) relapsed in addition to three patients in higher CR (n=7). Uni- and multivariate analysis showed that relapsing patients had higher TGFβ serum levels as well as lower percentages of IFNgamma-producing T cells before and early after transplantation. Furthermore, relapsing patients had a further decline in their thymic function between day 60-120 whereas nonrelapsing patients already showed increasing TREC values during this time interval. Collectively, patients who later relapse show a different pattern of immune reconstitution before and at early time points posttransplantation.

**P783**
**Early reconstitution of functional CMV immunity predicts a low risk for viral reactivation after HLA-haploidentical haematopoietic stem cell transplantation**
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Background: T cell-depleted HLA-haploidentical hematopoietic stem cell transplantation (haplo-HSCT) is characterized by a delayed T-cell reconstitution, which increases the risk of opportunistic infections. Among these, CMV reactivation syndromes are the major cause of non-relapse mortality during the first year after transplant. With the aim of accelerating immune reconstitution, different T cell-based strategies have been proposed. The development of these strategies is however limited by the current lack of validated T-cell biomarkers predictive of clinical events.
Aim: To identify T-cell biomarkers predictive of the risk of CMV reactivation as a "model" clinical event during the first year after T-cell based haplo-HSCT.
Results: In this study, we considered 89 patients undergoing haplo-HSCT and receiving polyclonal donor T cells either as delayed infusions (n=18) or in the form of an unmanipulated graft (n=71) followed by immune suppression with rapamycin. All patients were prospectively studied for polyclonal and CMV-specific T-cell biomarkers starting from day 30 until day 360. During this period, CMV

reactivation (>1000 copies per mL) was observed in 46 patients (52%) and CMV disease in 8 patients (9%), all treated according to guidelines. Polyclonal T-cell reconstitution was accelerated: at day 30, median CD3+ cells were 154 per µl (range 0-2146), CD4+ 52 (0-996), CD8+ 81 (0-1373); at day 90, median CD3+ cells were 378 per µl (0-2817), CD4+ 127 (0-804), CD8+ 173 (0-1922). Higher T-cell counts, however, did not associate with a lower risk of subsequent CMV reactivation. Differential counts of TNA, TCM, TEM or TEMRA cells, or a higher TCR spectratyping complexity score also failed to predict viral reactivation. Median CMV-specific IFN-g spots were 100 per mL (range 0-7400) at day 30 and 200 per mL (range 0-35,800) at day 90. At both time points, higher values of CMV-specific spots protected from viral reactivation (P<0.05). Interestingly, when CMV-specific spots were >1000 per mL, CMV reactivation was exceedingly infrequent (2% of all episodes, self-limiting).
Conclusions: Early reconstitution of functional CMV immunity protects from viral reactivation. A threshold of 1000 CMV-specific IFN-g spots per mL should be investigated as a surrogate marker in prospective trials.

**P784**

**CMV reactivation may protect against relapse in HLA-C KIR ligand group homozygous but not heterozygous recipients following allogeneic peripheral blood stem cell transplantation**

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Objective: Reactivation of cytomegalovirus (CMV) has been recognized to augment antileukemic mechanisms after hematopoietic stem cell transplantation (SCT). CMV has furthermore been shown to induce activation of non-licensed natural killer (NK) cells that have only non-self specific inhibitory killer immunoglobuline-like receptors (KIR). This may occur in case of HLA-C KIR ligand group 1/1 or 2/2 homozygosity of the recipient.
Methods: We have retrospectively analyzed the effect of CMV serology and CMV reactivation in 264 peripheral blood SCT recipients stratified by HLA-C KIR ligand status.
Results: Following transplants to HLA-C-group 1/1 or 2/2 homozygous recipients, CMV reactivation favourably influenced the cumulative incidence of relapse (28% *vs* 44% at 4 years; p=0.013) and the probability of progression-free survival (PFS; 44% *vs* 24%; p=0.015). In contrast, in HLA-C-group 1/2 heterozygous recipients, CMV reactivation had a rather detrimental effect on relapse incidence (33% *vs* 20%; p=0.17) and PFS (32% *vs* 43%; p=0.55). Vice versa, HLA-C group homozygosity was an adverse factor exclusively in recipients who never experienced CMV reactivation (relapse, 44% *vs* 20%; p=0.002; PFS, 24% *vs* 43%; p=0.003), and in CMV seronegative recipients (relapse, 40% *vs* 16%; p=0.03; PFS, 30% *vs* 51%, p=0.1).
Conclusion: These data suggest that reactivation of CMV per se does not exert an antileukemic effect after SCT, but rather enables graft-versus-leukemia effects specifically in HLA-C KIR-ligand group homozygous recipients who may otherwise have hyporesponsive NK cells due to a non-licensed state.

**P785**

**Recipient T-lymphocytes may selectively persist after successful myeloablative stem cell transplantation in children with acute lymphoblastic leukaemia and contribute to anti-viral immune responses**

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Introduction: Children with high-risk and relapsed acute lymphoblastic leukemia (ALL) are treated with allogeneic stem cell transplantation (SCT) following myeloablative conditioning. Monitoring of peripheral blood and bone marrow chimerism after SCT has been demonstrated to be a valuable parameter to predict leukemia recurrence. In patients with mixed chimerism, cessation of immune suppression and infusion of donor lymphocytes may result in elimination of residual recipient hematopoiesis, achievement of full donor chimerism and prevention of leukemia relapse.
Patients and Methods: To obtain further insight in chimerism profiles after myeloablative SCT in children with ALL (particularly performed in first and second continuous remission), we performed a retrospective analysis in a cohort of n=71 consecutive and evaluable ALL patients transplanted with (mis)matched family and unrelated donors between 2000-2010. Chimerism was routinely performed once in 2-4 weeks within the first 3 months and once monthly thereafter until 1 year postSCT in both peripheral blood mononuclear cell (PBMC) and granulocyte fractions.
Results: Within this cohort, n= 31/71 patients relapsed which was mostly preceded by mixed chimerism in both PBMC and granulocyte fractions. In the group of ALL patients with continuous complete remission (n=40/71), n=2/40 (5%) had persistent mixed chimerism (>5% recipient signal beyond day +60) which lasted 8 and >12 months, respectively. Mixed chimerism did not respond to tapering of ciclosporin A. Notably, repetitive analysis of bone marrow aspirates in these patients revealed complete donor chimerism and molecular remission. Detailed analysis of peripheral blood leukocyte fractions demonstrated that mixed chimerism was exclusively found within CD3+/CD4+ and CD3+/CD8+ T lymphocyte subsets. In both patients, the episode of mixed T lymphocyte chimerism coincided with adenovirus and cytomegalovirus reactivation, respectively. Adenovirus-specific T lymphocytes could be isolated and were found to be completely of recipient origin.
Conclusion: These data indicate that recipient memory T lymphocytes may resist successful myeloablative and anti-leukemic SCT conditioning and contribute to anti-viral immunity postSCT. Thus, prolonged mixed chimerism, when restricted to the T lymphocyte fraction, is not correlated with ALL relapse. In these particular cases, potentially harmful immunotherapeutic interventions to prevent suspected relapse may be avoided.

**P786**

**Changes of the dendritic cell distribution within the cornea and mucous membranes following HSC-Transplantation – visualized via *in vivo* confocal laserscanning microscopy**

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Objectives: *In vivo* confocal laserscanning microscopy (CLSM) is a new noninvasive method to visualize changes in cell distribution within tissues such as the cornea. Here, we investigated whether the distribution of dendritic cells (DC), seen as hyperreflective cells, is changing in epithelial cornea and oral mucous membranes after nonmyeloablative hematopoietic stem cell transplantation (HSCT). A correlation to the development of donor specific chimerism in peripheral blood mononuclear cells (PBMC) was evaluated.
Methods: After total body irradiation of 2 Gy, enriched HSC from canine bone marrow were transplanted in DLA-identical siblings by intra bone marrow application into the humerus and femur. As immunosuppressive drugs a combination of CSA (15 mg/kg BID; days -1 to +35) and MMF (20 mg/kg BID; days 0 to +27) was applied. PBMC samples for chimerism analyses were taken once before and then biweekly after HSCT. Chimerism was determined by a PCR of polymorphic nucleotide repeats using a capillary gel electrophoresis. On days -1, +28, +56 and +112 images of epithelial layers of canine cornea and of oral mucous membranes were taken by CLSM. The installation for the *in vivo* imaging consisted of the Heidelberg Retina Tomograph (HRT II) with the Rostock Cornea Module (RCM), which enables measurements of the ocular surface.
Results: Before HSCT, DC were rarely visible in the examined tissues. An increase of DC after HSCT was detected in each of the five dogs examined at one time point of measurement,

both in epithelia of cornea and of oral mucous membranes. The magnitude of increase and the time point of the highest DC density varied between the animals. After HSCT, an increase of donor derived PBMC was observed with peaks on d+28 in all five dogs (range 9.7 – 21.6 %). The two dogs with the highest donor chimerism showed the highest DC density in epithelia of cornea and oral mucous membranes, respectively. The increase in DC density occurred delayed in regard to the PBMC chimerism.
Conclusions: After HSCT, changes in DC distribution can be visualized *in vivo* in canine epithelia by the CLSM. In parallel to the development of PBMC chimerism, an appearance and increase of DC within the respective epithelium could be detected. The CLSM is a new excellent tool to visualize changes in cell composition within tissues after HSCT.

**P787**

**Low counts of plasmacytoid dendritic cells after engraftment are associated with higher early transplantation-related mortality in patients receiving unrelated haematopoietic stem cell transplantation**

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Background: The heterogeneous status of host immune defenses may influence the risk of infection and graft-versus-host disease (GVHD) after hematopoietic stem cell transplantation (HSCT). In such defense, dendritic cells (DC), which act as specialized antigen-presenting cells that bridge the innate and adaptive immune systems, are essential cell components.
Objectives: To monitor the recovery of different subsets of DC after unrelated umbilical cord blood (UCB), bone marrow (BM), and peripheral blood (PBSC) HSCT and to evaluate the impact of the distribution of these cell subsets on the outcome of the transplant.
Methods: DC [lineage negative, HLA-DR+ and CD123+ plasmacytoid (p) DC, CD11c+ myeloid (m) DC, and CD16+ monocytoid (mo) DC] were quantified by multiparametric flow cytometry at 6 sequential time points (at engraftment, and at days 3, 7, 14, 21 and 60 after engraftment). Overall, 34 patients (19 male; median age 13y, range 1-63y) receiving a UCB (n=15), BM (n=14) or PBSC (n=5) unrelated HSCT were studied. The most common diagnosis was acute leukemia (ALL, 12 cases; AML, 10; CML, 5; aplastic anemia/MDS, 6; Hodgkin lymphoma, 1; SCID, 1). Most patients received myeloablative conditioning (MAC) regimens (73%). Antithymocyte globulin (ATG) was used in 38% and total body irradiation (TBI) in 41% of cases. Median time to neutrophil engraftment was 18 days (range: 12-45). Median follow up time was 6 months.
Results: Patients who died from early transplantation-related causes (TRM) had significantly lower counts of pDC and mDC during the first 3 weeks after HSCT. At day 21 after engraftment, the median number of pDC and mDC was 0.9 and 2.0/uL among patients who died from TRM *vs.* 7.1 (p=.006) and 8.4/uL (p=.01) in the remainder, respectively. Patients presenting grade II-IV acute GVHD also had significantly lower pDC counts at days 14 and 21. There was no significant association of both the hematopoietic stem cell source and the conditioning regimen on the risk of TRM or acute GVHD.
Conclusion: Low pDC counts in the first weeks after unrelated HSCT are associated with an increased incidence of GVHD and mortality. The precise mechanisms that might explain the role of pDC on immunity early after HSCT deserve further investigations.

# Regulatory Issues

**P788**

**Operative protocol for assessing cryopreserved PBSCs quality: a single-centre experience**

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Introduction: It is known that biological and functional properties of peripheral blood stem cells (PBSCs) have a significant role in influencing the transplant outcome. Based on this consideration, we have established and applied an internal operative protocol of quality controls, with the aim of defining the graft validation, respecting international guidelines.
Methods: We established type and timing of the tests to perform during all phases of PBSC processing. We evaluated the parameters recognized as indicators of graft quality: total nucleated cells (TNC), mononuclear cells (MNC), cell viability, CD34+ cells count, colony forming unit-Granulocyte Macrophage (CFU-GM, Methocult Stem Cell Technologies), microbial and minimal residual disease (MRD) contamination. At collection: PBSCs, collected using a leukapheresis cell separator (Fresenius COM-TEC), are tested for microbial contamination. At Processing: assessment of TNC, MNC, CD34+(FacsCalibur Becton Dickinson;ISHAGE Protocol) and cell viability (7-AAD), CFU-GM, microbial and MRD contamination. After 30 days of cryopreservation and storage: revaluation of cell viability (trypan blue) on aliquots stored together with PBSC unit. This test is repeated immediately before transplant to verify the effective performance of the product. At PBSCs release: count of TNC, MNC, CD34+ (ISHAGE) and cell viability (7-AAD, trypan blue) and microbial contamination.
Results: From January 2010 to November 2011 we evaluated 175 autologous cryopreserved PBSCs, comparing the data obtained in all steps defined by our protocol with the final results of release. We observed a satisfying recovery for each parameter tested, in line with our expectations, and a reproducibility of the data at each time points. All data are shown as percentage of recovery: TNC 88,6% (r.62-100); TNC viability 69% (42-94); MNC 78% (r.50-93); CD34 71% (r.43-99). No case of microbial contamination.
Conclusions: Quality tests provided by our operative protocol allow us to evaluate the graft characteristics and to monitor all different steps of PBSCs production, from collection until distribution. An other important advantage is the possibility to keep critical points of the process under control.

**P789**

**Cell viability: a protocol of comparison and validation between cellular products and correspondent samples**

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Introduction: In the cryopreservation process of hematopoietic progenitor cells (HPC) the viability control after thawing has a crucial role. Such control is useful to confirm the validity of cryopreservation, storage and thawing processes. Therefore, we set up a internal protocol to determine how much the cell viability verified on the samples is representative of the correspondent cryopreserved HPC products. In our protocol, the viability must be available at HPC thawing time and carried out previously on an accompanying sample cryopreserved and stored in the same conditions of HPC product.
Methods: 109 HPC products and relative samples have been valued after thawing at 37°C to check the viability of total nuclear cells (TNC) using a vital test (7-AAD). The data have been analyzed using the independent sample test (T Test).
Results: The viability average on TNC was 76% (SD 17.6) on samples and 80,3% (SD 14,1) on HPC products. The HPC products' viability is confirmed by the clinical output. In fact, we have infused 4.9x10E6 (SD 1.3) CD34/Kg (average); the

engraftment of polymorphonucleated cells (PMN) (>100) has been at +10.9 days (SD 1.4) and of platelets (PLT) (>30.000) at +14.2 days (SD 0.1).
Conclusion: There are a few works in literature related to cell viability's validation on samples in comparison of correspondent HPC products. Our data show that the viability value (%) of the samples is significantly lower than respective HPC products.
Finally, we can affirm that cell viability of the samples can be a reliable and prognostic indicator of the viability recovery of respective thawed HPC products.

**P790**

**Analysis of expected versus real infused CD34+ value in cryopreserved PBSCs. A single-centre experience**

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Introduction: Cryopreserved autologous Peripheral Blood Stem Cells (PBSCs) are commonly used in treatment of malignant and non-malignant diseases. Transplant outcome, considered as achievement of a rapid and durable granulocytes and platelets recovery, is strictly related to the biological and functional properties of graft, particularly regarding its CD34+ count. An important aspect is to adequately cryopreserve and store PBSCs, guaranteeing their integrity until transplant.
Aim: To apply a standardized protocol for evaluating the main biological properties of PBSCs for predicting approximately, just at collection time, the real CD34+ count expected at transplant.
Methods: We analyzed PBSCs collected from 63 patients with different diseases (31 MM; 11 HD; 11 AL; 8 NHL; 2 MS), submitted to collection and autologous transplant from 2009 to 2011. PBSCs were obtained by leukapheresis with continuous-flow (Fresenius COM-TEC). Test and timing of our protocol are reported as follows. Collection and processing: count of total nucleated and mononuclear cells (TNC-MNC), CD34+ count (FacsCalibur, BD ISHAGE). At this step we calculated, using MNC% value, the probable CD34+ performance. At 30 days after cryopreservation and storage in liquid N2: cell viability (trypan blue) and evaluation of approximate CD34+ count expected at release. At transplant: after thawing and washing PBSC unit, we evaluated TNC, MNC, cell viability (tryoan blue and 7-AAD) and CD34+ count to definitively establish graft quality before releasing.
Results: All data are shown as mean value. At collection: TNC 180x10E8 (130-210); MNC 67,9% (r.40-87), CD34+ 4x10E6/Kg (r.0,54-7,4), CD34+ performance 2,7x10E6/Kg (r.0,38-5,2). At 30 days: TNC viability 67% (r.39-82), CD34+ performance 2,6x10E6/Kg (r.0,33-5,1). At transplant: TNC 158x10E8 (r.114-184); TNC viability 68,8% (r.42-92), CD34+ performance 2,8x10E6/Kg (r.0,37-5,8).
Conclusions: Our data demonstrate that just at collection time we are able to establish the effective performance of cryopreserved PBSCs, particularly referred to CD34+ count. We consider that this could be mainly explained by the close correlation existing among MNC collected, cell viability and CD34+ count calculated at transplant.

**P791**

**The effect of JACIE standards implementation on risk management in the JACIE accredited centre**

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Background: Risk management is the identification and assessment of every critical situation followed by coordinated application of resources to minimize, monitor, and control the probability and/or impact of unfortunate events. In a SCT programme, this assumes that the Quality Management System (QMS) is well established and set up to identify potential risks for patient and donor safety. Our center is JACIE accredited since 2008 for all facilities-Clinical Unit (CU), Apheresis Unit (AU) and Cell Processing Laboratory (CPL). These parts represent complex SCT transplantation programme with a single QMS. Methods:based on the analysis of adverse event reporting we retrospectively (2008-2011) identified some critical situation during the transplant process and determined the risk factors. According to the ISO risk analysis technique the Composite Risk Index (CRI) was established. For the CRI calculation following procedure was used: impact of the risk event (IR) x probability of occurrence (PO). IR and PO were assessed on a scale of 1 to 5 (1 and 5 represent the minimum and maximum possible impact of an occurrence of a risk). CRI thus took values ranging from 1 through 25, and this range was divided into three sub-ranges: Low (1-8), Medium (9-16) and High (17-25), depending on the sub-range containing the calculated value of the CRI. Results: during the years 2008-2011, we identified a total of 154 adverse events (73 in the CPL, 65 in the CU and 17 in the AU). In the CPL were assessed as Medium risk: lower number of CD34+ (16/73), labeling (7/73) and as Low risk: errors in documentation (3/73), technical problems (6/73), positive steritity testing (6/73). In the CU have been as Medium risk identified: errors in documentation (26/65) and as Low risk: technical problems (7/65), complication during transplantation (6/65), complications after platelet transfusion (4/65), pneumothorax after central venous catheter insertion (3/65). In the AU were assessed as Medium risk: compliacation during the apheresis (14/17) and as Low risk: trombocytopenia (3/17). During the analysis, we did not identify High risk potential hazard. Discussion: we applied ISO definied risk analysis technique to identify potencial critical steps in SCT programme. Based on the results of the risk assesment we evaluated the adequacy and effectiveness of how risks are identified and managed in the above areas.

**P792**

**Identification, categorisation and mapping of indicators used by JACIE-accredited stem cell transplantation programmes reveal an uneven distribution and coverage of processes**

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More than 145 European hematopoietic stem cell transplantation (HSCT) programs received JACIE accreditation since 2000, demonstrating compliance with FACT-JACIE international standards. The association of JACIE with improved patient outcome was recently documented. Conditions in which quality management systems were introduced and actual benefits remain to be fully evaluated.
The study explores one aspect of quality management: introduction and use of indicators. Through a questionnaire sent to JACIE accredited centers, we aimed at identifying indicators (name, domain of application, category, longevity and general description), understanding how they were set in place, whether or not similar indicators were used by different programs, and whether all of the HSCT processes were monitored. The survey was first sent to 14 French accredited HSCT centers and next to 68 other programs in 11 European countries. Categorization was double-checked against published criteria.
The response rate was 40% (32 programs). Two-hundred and ninety-three indicators were collected, including 224 (76%) that were introduced during the preparatory phase of JACIE accreditation. Indicators were associated with the following processes: measurement, analysis and improvement (54/293 or 18%); donor collection (49/293 or 16%); processing and storage of cell therapy products (37 /293 or 12.5%), administration of HPC (67/293 or 23%). Mapping reveals an uneven distribution of indicators across the different sub-processes that contribute to this highly-specialized medical procedure. Moreover, we found that only 101/293 indicators (34%) comply with the rules for implementation of a quality indicator, as defined by the FDX 50-171 standard.
This suggests that risks to donors / recipients are unevenly monitored, leaving critical medical steps with low levels of monitoring.

**P793**

**Predicting increases in bed capacity for haematopoietic stem cell transplantation or intensive chemotherapy through simulation modelling of ambulatory care**

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Aims: We and others have shown that it is feasible to treat selected haemato-oncology patients (pts) who need stem cell transplant (SCT) or intensive chemotherapy (IC) using an ambulatory care (AC) approach. In order to better understand the impact of AC on unit capacity we developed a computer model to simulate dynamic pt flows through our haemato-oncology unit when AC was used to treat a range of pts with haematologic cancer.

Methods: A simulation model was developed in Simul8 based on a hypothetical haemo-oncology unit with 9 HEPA filtered single rooms. Treatment pathways (pws) for pts needing SCT or IC for lymphoma, myeloma or AML consolidation were predefined, together with AC pws: i) all inpatient care, ii) ambulatory care in hospital sited flats, iii) ambulatory care at home. The model was populated with representative data from our unit, including pt numbers and treatment variables. In a model example, it was possible to perform a baseline of 92 SCT/IC treatments per year using conventional inpatient care pathways. In order to assess the impact of AC on this model, we assumed that 50% of pts needing SCT/IC would be suitable for AC, and that only those pts who lived within 30 minutes of the unit would be eligible for AC at home. We then ran simulations with varying degrees of AC.

Results: Despite a fixed number of HEPA rooms, our model predicted that 11-18 additional SCT/IC treatments per year would be possible with AC using 1-2 AC flats ± home care. Provision of a second AC flat had less impact than the one flat only, allowing only an additional 4 SCT per year, but with lower flat occupancy. AC with home care, in addition to a single AC flat, allowed an additional 7 SCT/IC per year.

Conclusions: Our simulation modelling predicts the availability of additional bed resource through using AC pathways in pts with haematologic cancer. Using this approach, it is also possible to determine the increased bed capacity predictions for specific treatment subgroups, including autologous and allogeneic SCT.

**P794**

**Cooperation of Dutch quality officers in haematology**

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Occasion: During EBMT 2011 quality officers in England and Scotland presented a deliberation structure that was well functioning. In the Netherlands there was also a need for adaptation and deliberation. Several quality officers took the initiative to check if adaptation and deliberation could be formed. Therefore a meeting took place in April 2011 with a small group of different institutes.

Goal: To achieve cooperation and deliberation between quality officers of the department Haematology in the Netherlands

Method: Quality officers were invited by e-mail for a meeting of orientation at June 1st 2011.

Results: On account of this meeting in April 2011 a decision was made to respond collectively to the DRAFT version of the FACT-JACIE International Standards 5th edition.

The first national meeting took place at June 1st, 2011. Quality officers from 6 different centres of transplantation took part in this meeting. Another 7 centres showed interest but were unable to attend the meeting at the specific date.

During the first meeting the differences within the draft version were discussed. Finally a joint response from 17 out of 19 centres was sent to JACIE. The joint response was also accorded with HOVON SCT group.

In accordance a discussion was held about the goals this group would like to achieve.

The three main items formulated are: to implement a uniform interpretation of JACIE standards, to share 'Best Practice' and to standardize processes/indicators.

In October 2011 a follow up meeting was held. The subjects to be discussed were: Comparison of a JACIE audit evaluation of 2 children hospitals, description of the process concerning donor trajectory and presentation of the quality system of the hospital attended.

At this meeting 11 quality officers were present out of 19 centres (adults and children).

Structure: meetings twice a year at different locations. Time span: 4 hours. Quality officer at location is required to be the chairman of the meeting. Coordination of the NKH will be performed by two members.

Conclusion: There is a need for cooperation between Dutch quality officers Heamatology. The two meetings held resulted in good output.

Discussion/Future: Inventories have been made concerning collaboration (HOVON *et al.*) Collaboration with these groups needs to be structured. Two meetings have been scheduled for 2012.

**P795**

**Challenges of setting up stem cell transplant centre in a resource-poor and developing country**

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Objectives: Hematopoietic stem cell transplantation(HSCT) is an approved therapy for malignant and non-malignant diseases. Nigeria with a population of 150 million like most developing countries requires HSCT that will be affordable and available.

Challenges include:

Lack of Trained personnel: Training of personnel at the Basel Hospital commenced in 2009 under the supervision of Prof. Alois Gratwohl but only a Hematologist, two Nurses, and a laboratory Scientist have been trained. There are plans for personnel from Nigeria to be trained in Switzerland and experts from switzerland to visit Nigeria.

Poor infrastructure/Equipment: four isolation room unit was built with hyperfilteration and high pressure single machines. However only basic equipment needed for Allogeneic transplantation are available with plans to procure equipment for autologous transplant. A generating plant with an inverter battery was provided as an alternative for power supply.

Selection of donor and patients: Sickle cell Anaemia (SCA) was the first group of patient selected for HSCT (first HSCT was 28th September 2011) because it accounts for 3% of Nigerian population and will attract funding from the Federal Government. HLA typing is currently done in Basel Hospital.

Conditioning: Reduced intensity conditioning (RIC) (FLU/BU) was used for our patient.

Stem Cell Harvesting: Bone marrow was the source of stem cell for the patient with a total of 800mls marrow plus 100mls anticoagulant. The unit had no equipment to separate CD34

**[P793]**

Table 1 Number of patients per year treated under different ambulatory care scenarios

| | All inpt care | 1flat | 2flats | 1flat+home |
|---|---|---|---|---|
| Total pts | 92 | 103 | 107 | 110 |
| Flat occupancy | - | 79% | 60% | 42% |

cells and patient developed a transient hypertensive crises with prolonged APTT controlled with Methyl Dopa and protamine
Supportive care: Supportive care in Nigeria is below international standards, and blood donation is mainly commercial. We recruited over 200 medical students as regular volunteer donors.
Blood products were transported in an ambulance with an inverter power battery connected to a small platelet vibrator and fridge for irradiation at a centre 300 km away from the Hospital.
Funding/Procurement of rare drugs: The first HSCT in Nigeria was fully paid for by Government at an estimated cost of 30,000 dollars (5million Niara). Most drugs for HSCT are restricted from Nigeria, expensive and not available.
Conclusions: In setting up a HSCT Unit in Nigeria the challenges are enormous but with the support from established Centres and a positive attitude by our Government, we could make this therapy available and affordable to most Africans who will require HSCT.

**P796**
**Assessing transplant outcome among Macedonian autologous recipients with haematological malignancies in a single centre**
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Several scoring systems are available for prediction of transplant outcome mainly for allogeneic recipients. A modified EBMT risk score and HCT-CI score was evaluated on autologous recipients in a single center experience.
Material and Methods: We stratified outcomes among 156 consecutive adult autologous recipients (35 MM, 20 NHL, 30HD, 9 ALL, 61AML, 1 Ewing). Median age was 35 years (7-63). In 40% of patients with lymphoproliferative diseases were with refractory/relapsed disease and other patients were in complete remission before SCT.
Results: HCT-CI risk was low in 19 (12%), intermediate in 42 (27%) high in 86(55%) and undetermined in 9 (6%). Two year OS was 45% (95%CI: 24-64%), 55% (95%CI: 40-68%) and 42% (95%CI: 24-64%) in the low, intermediate and high-risk HCT-CI groups respectively. Two year NRM was 36% (95% CI: 17-36%), 26% (95% CI 15-39%) and 30% (95% CI: 22-39%) in the low, intermediate and high-risk HCT-CI groups respectively. The multivariate analysis revealed that HCT-CI failed in prediction of OS and NRM but KPS (<90%) was a strong predictor of NRM as an independent predictor. According to the modified EBMT score 15 (9, 6%) patients had a score 0-3, 131 (83, 9%) had score 4-7, 10 (6, 4%) patients had score >8. OS two year was >80% in the low risk, 40% in the intermediate group and 25% in the high risk group.
Conclusion: HCT-CI and PS showed to be an effective tool in prediction of transplant outcome, mainly 2 year survival including variables that are independent to stem cell source, mobilizing regimen and conditioning. EBMT risk scores designed mainly for allogeneic transplant is still to be evaluated on antilogous recipients.

**P797**
**The regulatory issues concerning a university Cappadoccia centre**
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Erciyes University is located in the outskirts of Kayseri which has a population 942.000 people. Kayseri has a great historical background dating back to 2000 B.C. but its main frame was attained as a center of the Cappadocia region in the period of Romans, and took the name of Caesar-Kayseri.
Erciyes University Cappadoccia Bone Marrow Transplantation Unit was established in 1997 with 8 beds and now it is serving with a capacity of 39 beds in new and own transplant hospital building, with modern and last technology facilities; apheresis unit, processing laboratory.
Our center is the first applicant to the Jacie Accreditation in Turkey and has passed the inspection successfully and nowadays waiting for the final decision.
There has been 437 bone marrow transplants performed until 2009 and only in 2010 this numer has reached to 142 transplants.
According to the data of EBMT, the distribution of transplants performed in Europe in 2009 is showed on Figure 1.
The activity survey of Erciyes University transplant numbers are given in graph 1 and the distribution per each disease is given in graph 2.
Although this short time we have reached an immense success and we would like to continue this mission and improve ourselves furthermore.

## Stem Cell Source

**P798**
**No impact of donor non-specific anti-HLA antibodies on engraftment in unrelated cord blood transplantation in adult**
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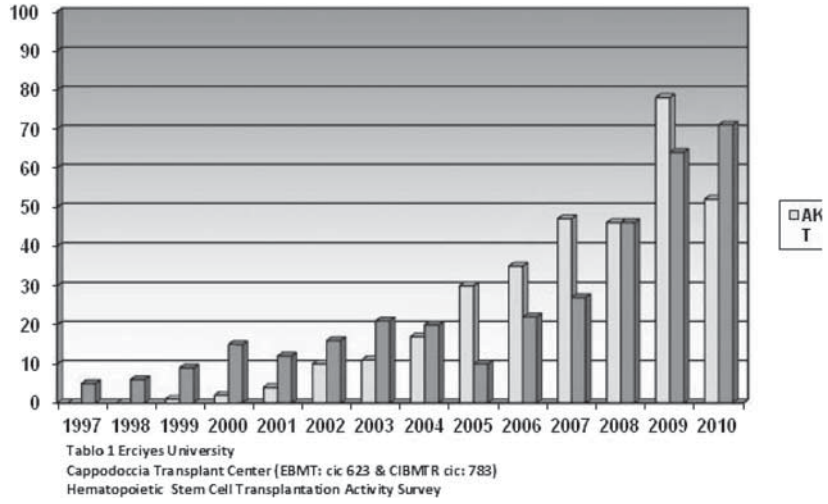
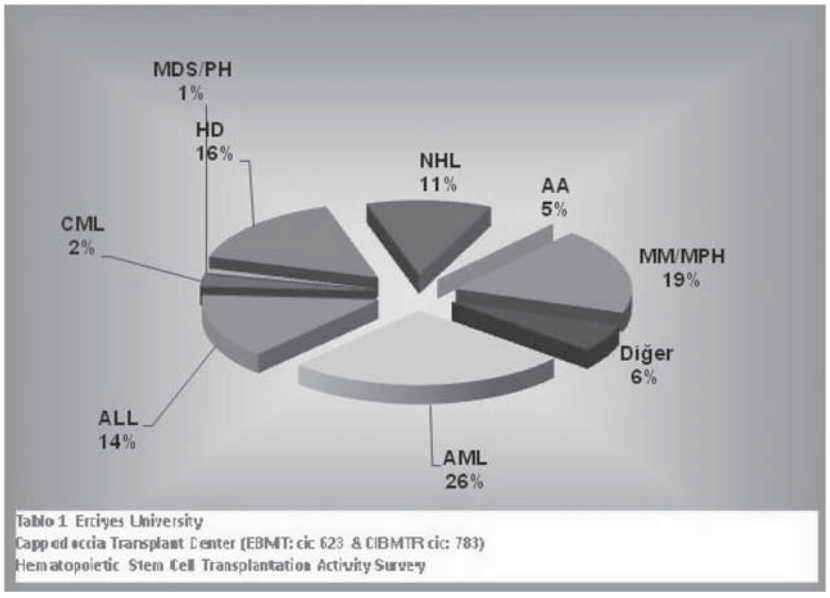
Background: Although donor-specific anti-HLA antibody (DSA) was clearly associated with graft failure in cord blood transplantation (CBT), clinical significance of anti-HLA antibodies without corresponding HLA antigens on cord blood (non-DSA) remains unclear. The present study evaluated the impact of non-DSA on engraftment in 176 CBT recipients.
Methods: From 2008, we prospectively screened anti-HLA antibodies before transplant to avoid selection of cord blood with corresponded to DSA in the recipient. Anti-HLA antibodies were tested using a Flow PRA method, and the specificity was identified by LAB Screen PRA and Single Antigen. Median fluorescence intensity of >1000 was considered to be positive.
Results: One hundred and seventy-six patients who underwent single CBT between 2008 and 2011 in our institute were included in this study. Their median age was 59 (range, 17-74). Overall, 60.7% were male, 88% were in high risk disease status, 94.8% were conditioned with Fludarabine-based regimen, and 82.3% received tacrolimus plus mycophenolate mofetil for GVHD prophylaxis. Median total nucleated cells and CD34+ cells infused were 2.56 (1.67-5.65) x $10^7$/kg and 0.91 (0.13-2.97) x $10^5$/kg, respectively. HLA disparities at antigen for HLA-A, -B, and -DR locus was 6/6 (n=2), 5/6 (n=31), 4/6 (n=133), and 3/6 (n=10). Anti-HLA antibodies were present in 64 of the 176 patients (36.3%) (ab-positive group). Among ab-positive group, 53 (82.8%) had antibodies against an HLA class I antigen, 9.3% against an HLA class II, and 7.8% against both class I and class II. Median number of the positive antibodies was 3 (range, 1-60). Cumulative incidence of neutrophil recovery at 42 after CBT was 81.2% for ab-positive group and 79.5% for ab-negative group (P=0.7). The type of antibodies (class I *vs* class II *vs* class I + II) and the number of antibodies (high; > 5 *vs* low; <5) had no significant influence on neutrophil recovery. In multivariate analysis, high degree of HLA disparities in host-versus graft direction (2-3/6 *vs* 0-1/6) was only significant negative factor for neutrophil recovery (Hazard ratio; 0.78, P=0.01). Even in the subgroup analysis of patients with high degree of HLA disparities, non-DSA had no effect on engraftment (p=0.3).
Conclusion: These results suggest that presence of non-DSA itself does not have any effect on engraftment.

[P797]




**P799**

**The collection and cultivation of umbilical cord derived cells for potential clinical use**

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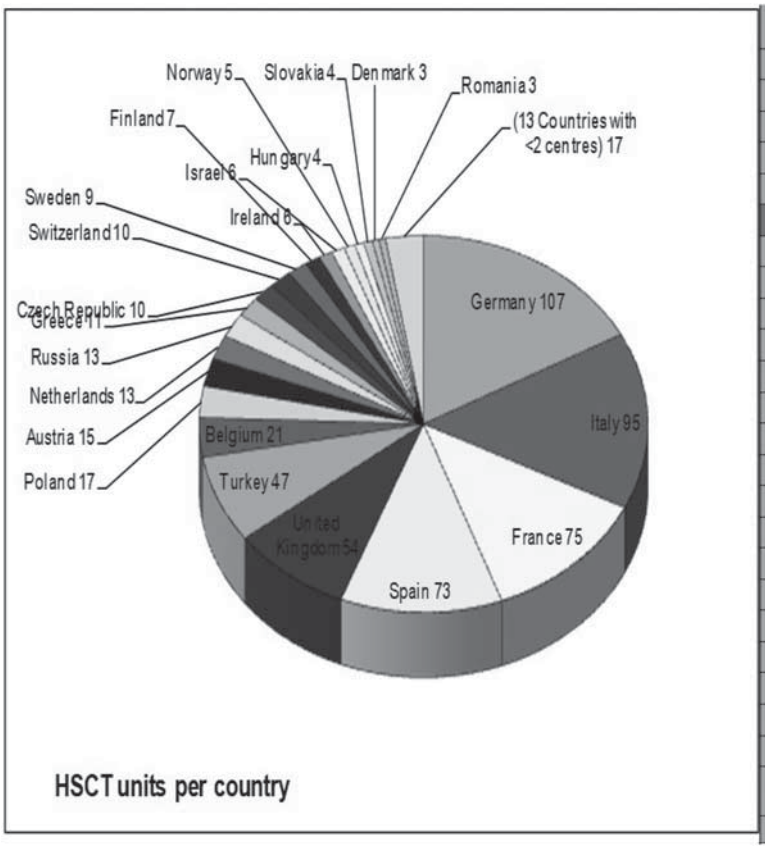
Introduction: Now a days, the mesenchymal stem cells (MSC) are new and giving new opportunities for therapies of various inherited and acquired disorders. Recently, the possibility of treatment of Graft versus Host Disease (GvHD) with MSC is the aim of intensive studies. MSC can be isolated from many sources. The most commonly used in clinical practice are bone marrow-derived cells, however their harvest is invasive for donor's health. Collection of UCB is easy, safe for both: newborn and mother, but it usually contains MSC in a very limited number. WJ as a source of MSC has all advantages of UCB and it is reach in cells which can be isolate, proliferate to a required number and cryopreserved. In the present publication we describe the results of collection, culture, investigation, and cryopreservation of cells derived from more than 500 UC collected by our group of stem cells banks (www.famicord.eu).

Material and Methods: UC were collected after natural deliveries as well as caesarian sections. After that WJ was isolated by mechanical dissection of cord's blood vessels. Plastic-adherent cells were isolated without enzymatic treatment and cultured in a MSC intended medium in the 37 °C, in the atmosphere of 5% CO2 in the air. Cells were enumerated and their viability was evaluated. Then the cells were cryopreserved in the presence of 10% DMSO and placed in the vapour phase of liquid nitrogen. The repeated cell counting, viability test, flow cytometric immunophenotyping, and functional *in vitro* differentiation assays were performed from thawed reference samples.

Results: We reported the low contamination level (less than 2%) of the UC tissue collected after both: natural deliveries and

[P797]




| COUNTRY | Activity Survey |
|---|---|
| Germany | 107 |
| Italy | 95 |
| France | 75 |
| Spain | 73 |
| United Kingdom | 54 |
| Turkey | 47 |
| Belgium | 21 |
| Poland | 17 |
| Austria | 15 |
| Netherlands | 13 |
| Russia | 13 |
| Greece | 11 |
| Czech Republic | 10 |
| Switzerland | 10 |
| Sweden | 9 |
| Finland | 7 |
| Ireland | 6 |
| Israel | 6 |
| Norway | 5 |
| Hungary | 4 |
| Slovakia | 4 |
| Denmark | 3 |
| Romania | 3 |
| (13 Countries with <2 centres) | 17 |
| | 625 |

caesarian section. The first adherent cells with fibroblast-like morphology were well distinguishable within a week after the initiation of the cell culture. The post-thaw viability was not lower than 85% and thawed cells can give rise the new culture within less than 4 hours. The immunophenotype (CD45-/CD34-/CD19-/CD14-/HLA-DR-/CD73+/CD90+/CD105+) was stable during the whole period of culture (15 passages). Cells were capable for differentiation into adipogenic, chondrogenic and osteogenic cells.
Conclusion: The described above results demonstrate a quick, repeatable and efficient method for WJ derived MSC isolation and propagation to get a “ready-for-use” unit containing MSC in a number suitable for potential clinical use.

**P800**

**The comparative outcomes of cord blood transplantation with bone marrow or peripheral blood stem-cell transplants from unrelated donors in patients with acute leukaemia: a single-institute analysis**

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Objectives: Our aim was to assess the leukemia-free survival (LFS) and some main results such as hematologic recovery, risk of graft-versus-host disease (GVHD), relapse and cytomegalovirus (CMV) infection after unrelated cord-blood translations compared with the outcomes of transplantations from the other unrelated graft source.
Methods: The clinical outcomes of 112 consecutive patients with acute leukemia and received umbilical cord blood (CBT) as a primary unrelated stem-cell source (n=38), or bone marrow transplant (BMT n=28, transplanted before Jan 2003) or peripheral blood stem-cell transplant (PBSCT n=46, transplanted after Jan 2003) during July 2000 and July 2008 were analyzed.
Results: Except for the patients were much younger in the CBT group (median age 10.5 in CBT, 30 in PBSCT, 20 in BMT), the other pre-transplant parameters such as gender, diagnosis, the phase of disease were comparative. All patients received myeloablative regimens mainly including BUCY; however, there were less anti-thymocyte globulin used for the BMT patients (3/38 without ATG in CBT, 0/46 in PBSCT and 18/28 in BMT). Significant delays in engraftment occurred after CBT for both white blood cell and platelet. The cumulative allo-engraftment rates were also significant lower (86.8% *vs* 97.8% *vs* 100% for WBC; 77.0% *vs* 97.5% *vs* 89.5% for PLT) for CBT. The incidence of II-IV aGVHD or III-IV aGVHD were much higher in the BMT group, but did not differ for the other groups (51%,13.2% *vs* 41%,10.5% *vs* 77.4%,41.5% for the CBT *vs* PBSCT *vs* BMT). The occurrence of extensive cGVHD was significant decreased for recipients with CBT (4%) compared with PBSCT (39.1%) and BMT (49.1%), although the rates of whole cGVDH were not reached statistically difference (30.3% *vs* 63.1% *vs* 60.1%). The patients had the similar rate of CMV infection (22/38 *vs* 28/46 *vs* 21/28), while the HC occurrence were lower after CBT (7/38 *vs* 16/46 *vs* 14/28). Till Nov 2011, there was no apparent difference in 5-year OS or LFS or relapse rate for each graft source (52.5% *vs* 52.5% *vs* 20.8% in CBT, 48.9% *vs* 46.7% *vs* 27.9% in PBSCT and 46.4% *vs* 42.9% *vs* 16.0% in BMT).
Conclusion: Our data showed that the OS, LFS and RR were similar among the three groups, although the hematologic recovery were lower and took longer in the CBT patients. The patients received CBT also had less occurrence of extensive cGVHD. These data support the use of umbilical cord blood donor as an alternative allogeneic donor as the unrelated adult donor.

**P801**

**Umbilical cord blood stem cell transplantation outcome**

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Objective: To review the results of umbilical cord blood transplantation in our center. Umbilical cord blood (UCB) due to the lower incidence of Graft-versus-Host Disease (GvHD) is an increasing available source of allogeneic hematopoietic stem cell transplantation in patients without suitably matched related or unrelated donor.
Methods: Fourty patients who received UCB transplantation (41 UCB units, one case of retransplantation) between 1998 and 2011 enrolled in the study. The male to female ratio of recipients was 26:14 with a median age of 5 years (range: 8 months-30 years). The most common diseases were major beta- thalassemia (10 patients; 25%), then acute lymphoblastic leukemia (8 patients; 20%) and severe combined immunodeficiency (SCID) (5 patients; 12.5%). Thirteen patients (31.7%) received UCB from human leukocyte antigen (HLA) matched-identical siblings, two (4.9%) from HLA-matched unrelated and 26(63.4%) from HLA mismatched unrelated. Five patients received double-unit cord blood transplantation (DCBT).
Results: The median number of post- thawing nucleated cells infused was $79\times10^7$/kg. The median number of CD34+ cells infused was $1.62\times10^6$/kg. The median number of CD3+ cells infused was $97.2\times10^6$/kg. Thirty patients had neutrophil recovery and 25 had platelet recovery till 100-day post transplantation. The median time to absolute neutrophil count recovery ($\geq0.5\times10^9$/L) was 21 days (range: 12-56). The median time to platelet recovery (count $\geq20\times10^9$/L) was 33 days (range: 19-62). The median follow-up time was 154 days. Ten patients (25%) did not engraft until day 100 after transplantation. Acute Graft versus Host Disease (GvHD) occurred in 20(50%) patients at median time of 11 days after transplantation. The most prevalent grade of aGVHD was grade III, in 9 (45%) patients. At present, 26 (65%) patients are still alive. Eight (20%) patients relapsed. The most common cause of death was infection. The six-month disease- free survival (DFS) and overall survival (OS) were 44.5% (SE: 9.2%) and 63.2% (SE: 8.5%), respectively.
Conclusion: Despite other studies, we found higher grade of aGvHD and graft failure among patients, which might be associated with a large number of mismatched alleles. Higher rate of infection observed because of delayed immune system reconstitution in recipients. Although the median time to engraftment is longer with using UCB but it is still a potentially available source for hematopoietic stem cells.

**P802**

**Safety and efficacy of unrelated cord blood transplantation with myeloablative conditioning for haematological malignancies: trial of Kanto Study Group for Cell Therapy**

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Background: Although unrelated cord blood transplantation (CBT) has been widely performed as a curative therapy for hematological malignancies, the optimal methods of transplantation including conditioning and GVHD prophylaxis have yet to be established. In this multi-institutional study, safety and efficacy of CBT for hematological malignancies using myeloablative conditioning were prospectively evaluated.
Patients and Methods: Patient eligibility was adult patients with AML, ALL, CML or MDS either lacking HLA-identical sibling donor and HLA-matched unrelated donor or requiring immediate allogeneic hematopoietic stem cell transplantation. Conditioning consisted of total body irradiation 12 Gy, cytarabine 2-3 g/m$^2$ every 12 hours for 2 days, and cyclophosphamide 60 mg/kg/day for 2 days as previously reported (Takahashi S, *et al.* 2004;104:3813). Continuous infusion of G-CSF (5 mcg/kg/day) was combined with cytarabine in patients with myeloid malignancies. Cyclosporine A and short-term methotrexate were used for the prophylaxis of GVHD.
Results: Thirty-three patients were enrolled and could be evaluated (Median age 37 years; range 21-54). Diagnoses were AML (n=20), ALL (n=6), CML (n=4), and MDS (n=3). Disease status at CBT was CR1 (n=12), CR2 or CP2 (n=5), and other advanced stages (n=16). Of the 33 patients, 31 achieved engraftment and 15 developed acute GVHD grades II-IV. With a median follow-up period 46.2 months (range: 31.0-65.8 months) in 16 surviving patients, 1-year non-relapse mortality rate was 15.2% (95%CI: 3.0-27.4). Causes of death were infection (n=4) and graft failure (n=1). Overall disease-free survival rates were 66.7% (95%CI: 40.0-93.4) and 50.0% (95%CI: 21.8-78.2) in standard-risk patients, and 42.3% (95%CI: 20.9-63.7) and 38.1% (95%CI: 17.3-58.9) in high-risk patients, respectively.
Conclusion: These results suggest that CBT can safely provide a high disease-free survival rate even in patients with high-risk hematological malignancies, and our transplant procedure could be one of the optimal methods of CBT.

**P803**

**Evaluation of immunomodulatory properties for human umbilical cord-derived multipotent mesenchymal stromal cells**

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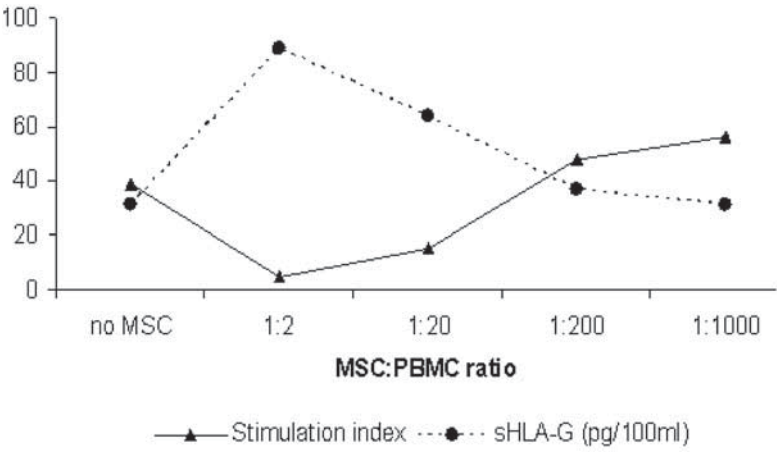
Current research is directed at identifying alternative sources of mesenchymal stromal cells (MSC) from ethically approved and readily available tissues. Human umbilical cord (UC) tissue provides a new source of MSC that exhibit differentiation and proliferation capacity. Several studies have indicated that MSC are immune privileged cells, unable to induce alloreactivity in humans. However, it has been documented that activated natural killer (NK) lymphocytes mediate MSC cytolysis, even though MSC can suppress NK-cell functions. The aim of the present study was to investigate immunomodulatory properties of human UC-MSC, with particular interest in their capacity to modulate lymphocyte proliferation as well as NK and cytotoxic T cell (CTL) activity. HLA-G surface expression and soluble (s)-HLA-G release were also investigated. Eleven UC, collected after obtaining informed consent from mothers of full-term infants delivered by caesarean section, were evaluated. For each sample, we were able to expand MSC. UC-MSC were characterized for plastic adherence, morphology, antigen surface expression, adipogenic and osteogenic differentiation potential. UC-MSC exhibited typical morphology and surface markers. Adipogenic and osteogenic differentiation potential was reduced compared to that reported for bone marrow derived MSC. Co-cultures of 5 UC-MSC lots with PBMC of the same healthy donor, at different MSC:PBMC ratios, were carried out in order to evaluate UC-MSC capacity to modulate PHA-activated lymphocyte proliferation, UC-MSC-directed cytotoxic activity, NK activity and CD3-redirected CTL activity. sHLA-G was quantified by ELISA in supernatants obtained under the same culture conditions. UC-MSC suppressed PHA-lymphocyte proliferation at 1:2 and 1:20 MSC:PBMC ratios, when high s-HLA-G levels were detected, while a moderate increase in PHA-induced proliferation was observed at lower ratios (representative exp in Fig). Three of 5 UC-MSC lots induced UC-MSC-directed cytotoxic activity; 5 of 5 UC-MSC lots suppressed NK activity and 4 of 5, CD3-redirected CTL activity. After co-culture, T lymphocyte subset number increased in 3 out of 5 exps, while NK cell number was reduced in all exps. Our results show remarkable variability in immune regulatory functions for UC-MSC; further studies are needed to define their use in clinical applications.

[P803]




**P804**

**Umbilical cord Wharton's jelly-derived mesenchymal stem cells: a potential cell source for infarct repair?**

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Background: Tissue adult stem cells are rare and their number and potency significantly decrease with age and concomitant diseases. These problems stimulate the search for alternative cell sources for infarct repair. The umbilical cord stroma can provide an attractive source since it contains a high number of fresh allogeneic mesenchymal stem cells (MSCs).
Objective: To examine whether MSCs from umbilical cord stroma are able to repair or regenerate the infarcted myocardium in rat.
Methods and Results: We developed a method that can be readily used to isolate and expand MSCs (Wharton's jelly cells) from human umbilical cord tissue. These cells display a fibroblast-like morphology, express mesenchymal markers, and have the potential to differentiate into osteogenic and myogenic cells. The *in vitro* study focused on their differentiation potential into cardiomyocytes using medium culture with 5-azacytidine. The *in vivo* study was performed in a rat model of MI: 7 days after MI, Wharton's jelly, bone marrow-derived MSCs (1*106 cells in 150µl sodium chloride) and saline were injected into the scar tissue, rats were injected with cyclosporine-A (15mg per 1kg) for a period of 30 days after cell transplantation. Serial echocardiography studies before and 60 days after injection showed that injection of Wharton's jelly stromal cells or bone marrow MSCs into a 7-day old infarct did not attenuate left ventricular (LV) systolic and diastolic dilatation and dysfunction. Postmortem morphometric analysis of the hearts showed a significant increase in wall thickness in the bone marrow MSCs treated group compared with control group (2.2±0.6 *vs.* 1.6±0.3, P=0.02).
Conclusions: The present work suggests that human MSCs (Wharton's jelly or bone marrow) transplantation does not prevent LV remodeling and dysfunction after MI in rat. However, further research is warranted to determine the optimal dose, timing and mode of delivery of Warrton jelly-derived MSCs for infarct repair.

**P805**

**Scheduled caesarean delivery should be preferred for collection of directed cord blood units due to the higher soluble HLA-G concentration**

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Human Leukocyte Antigen (HLA)-G is a non classical HLA class I gene which generates 4 membrane-bound (HLA-G1,G2,G3,G4) and 3 soluble (HLA-G5,G6,G7) protein isoforms by alternative splicing. A further soluble isoform (shedHLA-G1) is then produced by HLA-G1cleavage. During pregnancy HLA–G plays a major role in the maternal acceptance of the fetal allograft, downregulating uterine NK cells cytolytic activity and also inhibiting most T cell subsets response. For these tolerogenic properties, high soluble HLA-G (sHLA-G) levels have been correlated with better graft acceptance in patients undergoing solid organ transplantation and reduced incidence of Graft-versus-Host-Disease (GvHD) in patients undergoing hematopoietic stem cell (HSC) transplantation. Cord blood (CB) is collected at time of the infant donor's birth and its content of sHLA-G may be relevant for tolerance in the transplantation setting as CB is an alternative HSC source. Despite sHLA-G levels decrease in the second and third trimester of pregnancy compared to the first one, we formerly reported that detectable levels of sHLA-G can be found in CB plasma after delivery and also after cryopreservation and long term storage (data not shown). As labor has been reported to correlate with sHLA-G levels at the end of pregnancy, the aim of this study is to verify if the type of delivery influences sHLA-G content of CB. For this purpose we evaluated the concentration of G5 and shedG1 molecules by ELISA assay in cord blood (CB) plasma obtained from physiological pregnancies, all at term (>37 weeks), 24 CB units being collected after scheduled cesarean and 55 after vaginal delivery. Significantly higher sHLA-G levels were found in the group of CB obtained after scheduled cesarean (44 ng/ml) compared to vaginal delivery (35 ng/ml) (p=0.0462). While for unrelated HSC transplantation, plasma is routinely removed from CB units due to space saving for a cost-effective long term storage, directed CB units are cryopreserved as unmanipulated maintaining the beneficial of their sHLA-G content. In our hands labor may be the difference as CB derived from caesarean section contains more sHLA-G than CB derived from vaginal delivery. This may suggest that programming cesarean sections for collection of directed CB units could be of benefit, not only because of the increasing number of twin pregnancies and the relevance of logistics in this setting.

**P806**

**Low counts of CD4-CD8- T-cells during the first weeks after umbilical cord blood transplant**

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Background: CD3+CD4-CD8- T-cells can suppress alloimmune responses. However, the mechanisms by which these cells regulate immune responses and their role in the immune recovery after hematopoietic stem cell transplantation (HSCT) remain unknown.

Objectives: To compare the distribution of different subsets of T-cells and NK cells after unrelated allogeneic transplant in patients receiving umbilical cord blood (UCB), bone marrow (BM), or peripheral blood stem cells (PBSC).

Methods: T-cells (CD4+/CD8+/CD4-8-/CD4+8+), and NK cells subsets (lineage negative and 56++16-/56+16++) were quantified by multiparametric flow cytometry at 6 sequential time points (at engraftment, and at days 3, 7, 14, 21 and 60 after engraftment). Overall, 34 patients (19 male; median age 13y, range 1-63y) receiving a UCB (n=15), BM (n=14) or PBSC (n=5) unrelated HSCT were studied. The most common diagnosis was acute leukemia (ALL, 12 cases; AML, 10; CML, 5; aplastic anemia/MDS, 6; Hodgkin lymphoma, 1; SCID, 1). Most patients received myeloablative conditioning (MAC) regimens (73%). Antithymocyte globulin (ATG) was used in 38% and total body irradiation (TBI) in 41% of cases. Median time to neutrophil engraftment was 18 days (range: 12-45). Median follow up time was 6 months.

Results: As compared to BM/PBSC, UCB was associated with a delayed neutrophil engraftment (28 days *vs.* 17 days; p=.01), and a trend to lower counts of all T-cell in the first 3 weeks. At day 14 after engraftment, the median number of total T CD3+ cells was 149/uL for UCB *vs.* 828/uL for BM/PBSC recipients (p=.0004). The median number of CD4-CD8- T-cell was significantly lower in UCB recipients as compared to BM and PBSC at all times during the first 3 weeks (median 2/uL *vs.* 22/uL at day 3, p=.001; 2/uL *vs.* 13/uL at day 7, p=.003; 3/uL *vs.* 27/uL at day 14, p=.005; and 3/uL *vs.* 45/uL at day 21 after engraftment, p=.04). At day 60, Total T and CD4-8- cells counts were comparable between UCB and BM/PBSC recipients (11/uL *vs.* 30/uL, p=.13). No significant differences were observed between both groups as regards the distribution of NK cells subsets at any time. There was no significant influence of MAC, ATG or TBI on the differences in T-cell counts observed between the two groups.

Conclusion: UCB recipients have lower counts of CD4-/CD8- T-cells during the first weeks after HSCT as compared to BM or PBSC recipients. The impact of this finding on the transplant outcome remains to be determined.

**P807**

**Novel stem cell enumeration method by flow cytometry shows concordant results across four study sites**

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The accuracy evaluation for the BD™ Stem Cell Enumeration (SCE) Kit was conducted at four clinical sites using flow cytometry to enumerate the CD34+ cells, a stem and progenitor cell marker in leucocyte populations (CD45+). The objective was to evaluate the concordance between two investigational methods using the BD FACSCanto™ II (BD SCECanto) and BD FACSCalibur™ (BD SCECalibur) systems and a predicate method (Beckman Coulter Stem-Kit). Study testing was performed on 1,032 delinked and leftover enrolled specimens from clinical flow cytometry testing. The number of analyzed specimens for BD SCE Canto were 918, and for BD SCE Calibur were 905. Specimen types were normal and mobilized blood, frozen and thawed bone marrow, and leukapheresis and cord

**[P807]**

Figure 1. Bias Analysis

| BD SCECanto vs BCI Stem-Kit | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Absolute Difference | | | Relative Difference | | |
| Variables | Bin | N | Mean Bias | SD | 95% CI | Mean % Bias | SD | 95% CI |
| Viable CD 34 | Low | 167 | -0.1 | 3.1 | (-0.5, 0.3) | NA | NA | NA |
| | Mid | 496 | -0.7 | 8.9 | (-1.36, -0.035) | -1.6 | 19.4 | (-3.1, -0.2) |
| | High | 255 | -1.4 | 28.7 | (-4.3, 1.6) | -1 | 13.5 | (-2.4, 0.39) |
| % CD34 in CD45 | Low | 512* | -0.004 | 0.1 | (-0.01, 0.0002) | -2.2 | 29.2 | (-4.4, -0.1) |
| | High | 406 | 0.078 | 0.5 | (0.04, 0.12) | 2.1 | 27.2 | (-0.11, 4.3) |
| Viable CD45 | All | 918 | NA | NA | NA | -1.7 | 21.2 | (-2.8, -0.5) |

| BD SCECalibur vs BCI Stem-Kit | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Absolute Difference | | | Relative Difference | | |
| Variables | Bin | N | Mean Bias | SD | 95% CI | Mean % Bias | SD | 95% CI |
| Viable CD 34 | Low | 156 | -0.2 | 1.4 | (-0.4, -0.05) | NA | NA | NA |
| | Mid | 492 | -0.6 | 9.6 | (-1.3, 0.2) | -0.9 | 18.7 | (-2.3, 0.5) |
| | High | 257 | -3.6 | 31.5 | (-6.8, -0.3) | -1.7 | 13.9 | (-3.1, -0.2) |
| % CD34 in CD45 | Low | 487 | -0.002 | 0 | (-0.01, 0.001) | -0.8 | 28.8 | (-3.0, 1.3) |
| | High | 418 | 0.166 | 0.9 | (0.09, 0.24) | 3.2 | 28.3 | (0.9, 5.5) |
| Viable CD45 | All | 905 | NA | NA | NA | -2 | 25 | (-3.4, -0.6) |

*Note: On the predicate system 11 samples (DRK241, DRK242, DRK322, DRK323, DRK 325, DRK 331, DRK400, DRK289, DRK290, RPC192, RPC022) have a value of 0 for viable CD34 and %CD34, therefore N=501 for the relative difference calculation of %CD34 Low Bin.

blood anticoagulated with CPD, ACD-A, heparin, and EDTA alone or in combination. Fresh leukapheresis was used to show site equivalence on procedures for sample preparation, testing and analysis; the mean relative bias showed agreement within the predefined criteria for BD SCECanto (-2.81 to 4.31±7.1) and BD SCECalibur (-2.69 to 5.2±7.9). Data were analyzed and reported as absolute and relative differences compared to the predicate for viable CD34+, percentage of CD34+ in CD45+, and viable CD45+ gates. Bias analyses were performed based on distribution of predicate values in low, mid, and high bins using optimal gating for BD SCECanto and manual gating for BD SCECalibur. Values for viable CD34+, percentage of CD34+ in CD45+, and viable CD45+ in the different bins from both investigational methods were presented as absolute and relative differences. Results from both investigational methods met predefined acceptance criteria. Deming regression analyses showed an R2>0.92 for both investigational methods. In conclusion, the results for the accuracy study met the acceptance criteria in both investigational methods, demonstrating concordance to the predicate method for absolute viable CD34+, percentage of viable CD34+ in CD45+, and absolute viable CD45+ populations (or gates).

**P808**

**Inferior viable CD34 recovery in cryopreserved allogeneic compared to autologous HPC, apheresis products**

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Allogeneic haemopoietic progenitor cells collected by apheresis (HPC, Apheresis) are cryopreserved less often than autologous harvests. In recent years, cryopreservation of allogeneic HPC has been performed more frequently to store excess HPC or T cells for donor lymphocyte infusions or to circumvent issues of donor availability / product transport, particularly in light of recent worldwide events (Sept 11, swine flu and flight disruptions due to volcanic ash).
During 2006 to 2009, 30 allogeneic and 349 autologous HPC, Apheresis products were cryopreserved in this laboratory. It was noted that the post-thaw viable CD34+ recovery was lower in the allogeneic (median = 63%, range 16-92) than autologous products (72%, 13-151; p<0.0004). Hence this study aimed to determine factors that influence post-thaw CD34 recovery.
Univariate analysis demonstrated weak inverse correlations between viable CD34 recovery and the collection to freeze time interval (Spearman r=-0.10, p=0.048), frozen nucleated cell concentration (NCC; r=-0.17, p<0.0001) and neutrophil content (r=-0.19, p=0.0008). Multiple regression analysis demonstrated that collection to freeze interval (p=0.006), neutrophil content (p<0.0001) and allogeneic donors (p<0.001) significantly affected viable CD34 recovery, but NCC did not (p=0.14).
Nine of the cryopreserved allogeneic products have been infused, with patients attaining an absolute neutrophil count of >0.5x109/L in a median of 18 days (range 11-31) and platelets >20x109/L in 27 days (14-58), which is not significantly different to that observed following infusion of 133 fresh HPC (neutrophil median 17 (9-45) days, p=0.9; platelets median 18 (10-101) days, p=0.2).
This data indicates that the lower post-thaw viable CD34 recovery in allogeneic HPC, Apheresis may be due to intrinsic properties of allogeneic donors in addition to the higher neutrophil content and more prolonged pre-cryopreservation storage periods for these products. Post-thaw analysis of viable CD34+ content is recommended to ensure sufficient viable CD34+ to facilitate engraftment.

**P809**

**Post-thaw viable CD34+ cells enumeration and engraftment in autologous stem cell transplantation**

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High dose chemotherapy followed by autologous stem cell transplantation represents an important therapeutic option in numerous malignant diseases. The number of CD34+ cells infused is considered to be a predictor of hematopoietic engraftment. The aim of this study is to evaluate if CD34+ cell dose measured post-thaw may represent a better predictor of engraftment than CD34+ cell dose evaluate pre-freezing.
Total and viable CD34+ cells were counted using single platform flow cytometry and viability dye7-amino actinomycin D (7-ADD) before freezing and afterthaw. Stem cells were cryopreserved in 10% dimethylsulfoxide (DMSO).
113 consecutive peripheral stem cells autologous transplantations performed between November 2009 and November 2011 in 99 patients (pts) affected from hematological malignancies were analyzed. Patients' median age was 56 years (range 18-67); 14 were double transplants; 57 pts were affected by multiple myeloma, 27 by non-Hodgkin lymphomas and 15 by Hodgkin lymphoma. Melphalan 200mg/$m^2$ was the conditioning regimen utilized in 72/113 procedures, BEAM in 38/113 and FEAM in 3/113. Granulocyte colony stimulating factor (G-CSF) was administered to all pts from day +6.
The median number of CD34+ re-infused cells evaluated pre-freezing was 5,94x106/kg (range 1,99-13,49) versus 2,86 x106/kg (range 0,33-7,80) post-thaw. Only in 4/113 transplants (4.5%) infused CD34+ cells were less than 2,5x106/kg at pre-freezing evaluation while in 60/113 (68%) they showed to be less then 2,5x106/kg at post-thaw evaluation.
Median neutrophil engraftment (>0.5x109/l) was 12 days (range 8-21) and median platelet engraftment (>20 x109/l) was 14 days (range 9-49).
Using Cox regression analysis, only the number of CD34+ cells evaluated prior to re-infusion (post-thaw) significantly correlated with neutrophil (p .00) and platelet engraftment (p .001).
Even if no standardized method for thawed CD34+ cells enumeration has been so far assessed, this data may indicate that estimation of viable CD34+ cells at re-infusion may be a better predictor of engraftment than the CD34+ cells count before freezing.

**P810**

**Cell subtypes dose impact on engraftment, chimerism, graft-versus-host disease and survival after allogeneic haematopoietic stem cell trasplant**

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Introduction: The influence of graft composition on the clinical outcome of allogeneic hematopoietic stem cell transplant (HSCT), including CD34+ cell dose, remains controversial. Aim: To analyze the influence of graft cell subset composition on allogeneic HSCT clinical outcome.
Patients and Methods: Retrospective study of 115 consecutive patients undergoing HSCT between January 2005 and January 2011 in our center. The dose of cell subpopulations of the infused graft: total nucleated cells (TNC), CD34, CD3, CD4, CD8, CD19 and CD56 was analyzed and correlated with engraftment, chimerism, incidence of graft versus host disease (GVHD), relapse and overall survival.
Results: The median age was 42 years (range 10-69) and 58% (n=68) were male. Forty seven percent (n=54) received myeloablative conditioning, 68% (n=78) received progenitors from related donor and 97 patients (85%) received peripheral blood progenitor, 10 (9%) bone marrow and the rest umbilical cord blood progenitors. The median CD34 infused was 4.19 x $10^6$/kg

**[P810]** Table 1. Details on the distribution of the different cell subsets infused.

| | CNTxkg | CD3xkg | CD4xkg | CD8xkg | CDN56xkg | CD19xkg |
|---|---|---|---|---|---|---|
| Median | 9,155 | 2,365 | 1,29 | 0,7 | 0,24 | 0,4 |
| Minimum | 0,057 | 0,005 | 0,001 | 0,001 | 0,001 | 0,001 |
| Maximum | 32,53 | 6,06 | 3,49 | 1,84 | 1,45 | 1,89 |

(range 0.06-32.5) (other cell subsets are detailed in Table 1). In univariate analysis, infusion of higher doses of TNC, CD34, CD3, CD56 and CD19, was significantly associated with faster neutrophil engraftment (≥ 0.5x10$^9$/L) (p 0.011; 0.016; p 0.012; p 0.031; p 0.012 respectively) and faster lymphocyte engraftment (≥0.1x10$^9$/L) (p<0.001, 0.016, 0.003, <0.001 and 0.003 respectively). The platelet engraftment (>20 x10$^9$/L) was significantly related only with CNT, CD34 and CD56 dose infused (p<0.001, 0.004 and 0.004 respectively). There was a significant correlation among complete chimerism at day +360 with higher CD3+ cells dose infused (p=0.014). Patients who achieved neutrophil engraftment before day +10 had a significantly higher overall survival (p 0.037). However, the multivariate analysis did not confirm any significant association between cell dose infused and the incidence of GVHD, relapse of the underlying disease or survival.
Conclusions: This study confirms that not only infusion of higher doses of CD34+ and TNC are followed by a faster hematologic engraftment, but also the infusion of higher dose of CD56+, CD19+ and CD3+. Interestingly, the dose of CD3+ infuse is significantly correlated with a higher rate of complete donor chimerism on day +360. However, we cannot demonstrate significant relationship between the studied cell subsets dose infused and the incidence of GVHD, disease recurrence or survival.

**P811**
**Mobilization of autologous peripheral blood stem cells with Spectra Optia v5.0: a single-centre experience with an automatic interface-controlled apheresis system**
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Available manual apheresis systems generally produce stem cell yields of consistently high quality that can be safely used for autologous transplantation. However, manual apheresis needs continuous interface monitoring/adjustement, suffers from interface instability in poor mobilizers, high collection variability, high platelet loss and failure to electronically document parameters. A new advanced apheresis system, Spectra Optia v5.0, was designed to override these disadvantages.In our study, we evaluated data of 10 stem cell leukaphereses performed in 8 patients with various malignancies using Spectra Optia during 2011 to test its feasibility and effectiveness. We compared our data with those obtained from 225 patients that had undergone a stem cell collection for autologous transplantation in our Department between 2004 and 2011, using the COBE Spectra machine. The use and function of automatic interface control of Spectra were satisfactory. Due to the application of lower inlet volumes/min, as compared to corresponding volumes with the COBE Spectra machine, our apheresis with Spectra Optia usually took a longer time (median 447 min versus 317 min, p<0.005). Regarding other collection parameters, such as the percentage of CD34+ cells in the final leukapheresis product, total yield of CD34+ cells, and product volume data were comparable for both devices (0.74% versus 0.97% CD34+, p=0.103; 6.85 versus 7.1x10$^6$ CD34+/kg, p=0.752; and 403.5 versus 353 ml graft volume, p=0.094, respectively). Time to engraftment was also comparable for both apheresis devices. Time interval to neutrophil counts >500/µl, neutrophil counts >1500/µl did not significantly differ (10 days versus 9 days, p=0.386 and 11 days versus 10 days, p=0.229, respectively). However a delay in platelet recovery with Optia device need to be confirmed with additional data from apheresis procedures (12 *vs* 11 days to platelet counts >25000/µl, respectively; p=0.037). Platelet loss with Optia was less than with COBE Spectra (1278 versus 2415 x 103/µl, p=0.014). No significant differences were observed for product hematocrit between Optia and COBE Spectra (5.7% versus 6.6%, p=0.392).In conclusion, the automatic Spectra Optia aphereses were associated with similar and equally variable stem cell collections as aphereses with COBE Spectra. Further data are needed to clarify the potential benefit of lower platelet loss using Optia. Updated results will be presented in the meeting.

**P812**
**Efficacy and quality of stem cell mobilisation as well as storage in a single centre**
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Introduction: Autologous stem cell transplantation (ASCT) is currently the standard treatment for various haematological malignancies. We would like to evaluate the efficacy and quality of stem cell mobilization as well as storage in our centre for the last 5 years.
Method and Results: We retrospectively analyzed a total of 404 patients between January 2007 and December 2011 who underwent peripheral blood stem cell (PBSC) mobilization, using G-CSF with chemotherapy mainly consists of high dose cyclophosphamide or etoposide. Majority were lymphoma patients (Non-Hodgkin's lymphoma (NHL) 166; 41.08% and Hodgkin's disease (HD) 105; 25.99%), followed by multiple myeloma (MM) 70; 17.33% and acute leukaemia (acute myeloid leukaemia (AML) 57; 14.11% and acute lymphoblastic leukaemia (ALL) 6; 1.40%). There was no significant difference in gender distribution but the age distribution was highly influenced by the type of disease with MM represented the oldest group (median age 54 year old) and HD the youngest group (median age 24 year old) respectively. We mobilized patients based on peripheral blood CD34 count (PB-CD34) cutoff points at least 10/uL; and with this criteria, 341 (84.41%) patients were successfully collected a minimal target cell dose of 2x10$^6$ cells/kg with the most successful groups being MM (98.48%) and HD (92.78%) as compared to others (NHL 90.08%; AML 88.10%; ALL 80%). Most of them achieved the target cell dose after first apheresis (MM 84.62%; HD 78.89%; NHL 71.55%; AML 67.50%; ALL 60%). The collected stem cells were immediately sent to our stem cell laboratory for cryopreservation using 5% DMSO. The mean of stem cells viability prior cryopreservation was 99.45% (range: 92.72%-100%) and post thawing was 96.88% (range: 91.0%-99.0%) respectively. There was no documented microbial contamination in the processed stem cell for the last 5 years except very occasional fibrin clot reported after thawing

(<1%), in particular among those collection with very high cell count and platelet. Most of the cryopreserved stem cells were reinfused within 3 to 4 months after harvesting. The average neutrophils and platelet engraftment was 10.9 days and 12.8 days respectively among 223 harvested patients who underwent ASCT successfully.
Conclusion: We managed to achieve promising and successful stem cell mobilization and storage program especially among MM and HD cases. There is still room for improvement among NHL patients.

**P813**
**Autologous transplantation of peripheral blood stem cells mobilised by cyclophosphamide 6 g per bsm and pegphilgrastim 18 mg**
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Background: The use of G-CSF is most widespread method of peripheral blood stem cells (PBSC) mobilization during past decade. This cytokine may be applied alone or in combination with chemotherapeutic agents. When pegfilgrastim became available, great interest appeared to replace multiple injections of conventional form of G-CSF by single injection of pegilated form.
Formerly we had estimated mobilizing activity of pegfilgrastim given as single dose of 18 mg following chemotherapy (CT). Our experience demonstrated that combination CT with pegfilgrastim for PBSC mobilization is safe and effective. Cyclophosphamide 6 g per b.s.m. (CY6) followed by pegfilgrastim, single dose of 18 mg (Peg-F18), shows high CD-34+ cell PB peaks, laid in restrict, well predictable time.
Now we take an attempt to estimate potency of hemopoietic reconstitution after autologous transplantation (AutoT) of PBSC mobilized by CY6 and Peg-F18. The aims of our research were: to estimate the time of neutrophyl recovery and transfusion-dependent thrombocytopenia resolution.
Methods: Nineteen consecutive cancer patients (pts) were treated from July 2010 to December 2011. There were 7 female and 12 male, age was 20–53 y.o (median 48). Diagnoses were: aggressive lymphoma (6), multiple myeloma (6), poor-risk Hogkin lymphoma (7). They all were mobilized by CY6+ Peg-F18 regimen. Then AutoT of received material were performed. It is important to note, that all pts were in the phase of first partial response to “first line” CT. Indication for AutoT was: to achieve the complete response. Conditioning regimens were following: Melphalan 200 mg per bsm (multiple myeloma), CBV (lymphoma), BEAM (Hogkin lymphoma). The PBSC dose estimated by CD34+ cells consisted of 3,0 – 16,3 (median 4,6) million per kilo b.w. Every pt received pegilated G-CSF 6mg the next day after AutoPBSC infusion.
Results: There were no transplant-related deaths in our group. Day of AutoT marked as “day 0”. Neutrophyl recovery (more, then 0,5 thousand per microliter) was registered on day 8-17 (median 10). Transfusion-dependent thrombocytopenia was resolved on day 6-17 (median 11). We did not observe transplant failure in our group.
Conclusion: Our experience demonstrates that combination CY6+ Peg-F18 for PBSC mobilization allows to collect the material, suitable for further AutoT. The hematologic recovery was fast and sustained. However, this group is good-risk selective. This mobilizing regimen needs further investigation.

**P814**
**Plerixafor *ex vivo* mobilization of placental-derived haematopoietic stem cells**
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The placenta is a rich source of CD34+ cells which may not be fully mobilised using current cord blood collection techniques. The stem cell dose is the most important factor for determining speed of engraftment and outcome in haematopoietic stem cell transplantation. A modified two-step collection technique in which the initial collection is followed by placental perfusion with 50 ml heparinised 0.9% saline has shown a 15% increase in the number of nucleated cells. Our study aim was to assess whether plerixafor could enhance recovery of CD34+ cells and change the immune profile of the perfusate. Ten cord blood units were collected as per our standard operating procedures. After cord blood collection, 5 placentas were perfused with 50 ml heparinised 0.9% saline by slow infusion over 50 minutes and perfusate collected after 5 minutes of starting the infusion over 50 minutes (controls). The other 5 placentas were treated the same way except for the addition of 24 mg Plerixafor to the 50 ml heparinised 0.9% saline (plerixafor arm). The samples were analysed by multicolour flow cytometry using a combination of antibody cocktails to identify and enumerate CD34+ stem cells, T cells, T cell precursors, T cell subsets, T regulatory cells, B cells, NK and NK cell precursors, and dendritic cells. The CD34+ cells were further quantified by CFU. Successful recovery of perfusate was achieved from 9 placentas (mean volume 44 ml). Percentage increase in numbers of cells recovered by perfusing the placenta was calculated from total numbers of CD34+ and CD45+ cells collected from each cord and placenta pair. There was no significant difference in the perfusate volumes (48ml v 38ml, P=0.152) or recovery of CD34+ (5.06% v 5.79%, p=0.74) or CD45+ (4.69% v 2.61%, p=0.74) cells from the placenta between the 2 arms. The number of progenitor cells was similar between the cords and the perfusates. Viability of haematopoietic stem cell progenitors by CFU was also similar in the two arms. Clonal efficiency between the 2 groups was similar (unpaired t test, p=0.2). No statistically significant differences was found in the CD3, CD4, CD8 T cells, Tregs, NK, DCs, progenitor T and NK cells between the 2 groups. In this pilot study plerixafor did not significantly increase the CD34+ stem cells and other cell subsets. It is possible that the duration of infusion was too short to mobilise the stem cells. Further studies are warranted to investigate this further.

**P815**
**Post-thawed analysis of cryopreserved haematopoietic progenitor cells using a semi-automatic cell processing**
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Background: Toxicity caused by direct transfusion of thawed hematopoietic stem cells (HSCs) is related both to the amount of dimethyl sulphoxide administered, as well as to the infusion of debris and the release of intracellular substances. To minimize these effects, some centers use automatic washing, although this procedure can result in cell loss.

Objectives: Evaluate the effectiveness of the semi-automatic HSCs washing process (Cobe 2991) used in our center in terms of the side effects of infusion, cell recovery and microbial contamination.
Patients/Methods: Between April 2003 and January 2011 there were 256 HSCs washing processes for autologous transplantation. Until June 2009 thawing was performed in a bath at 37°C (n=181) and then in a dry heat bath (n=75). 25% of the freezing process (n=64) had a frozen process volume >400 ml (range 100-1100) and a freezing time >3 months (range 0,2-92). The variables studied were the following: total nucleated cells (TNC), CD34+ cells, viability, absolute neutrophil counts (ANC), microbial contamination and adverse effects (AE) of the infusion.
Results: The median volume infused was 197 ml (range 70-473). The median pre and post-processing TNC was 5,6 and 4,4x$10^8$/kg respectively, recovery of 80%. The median pre and post CD34+ cells was 3,2 and 3,1x$10^6$/kg respectively, with a recovery of 94%. The viability post wash was more than 75% to 90% of the products. There was no relationship between cell recovery and freezing time. The median ANC reduction in pre and post-process product was 70%. The median TNC in the stock liquid waste was 0,02x$10^8$/kg. Microbial contamination was detected in 11% of the processes (n=25: 18 coagulase negative Staphylococcus (CNS), 1 Acinetobacter iwoffi, 1 Alcaligenes sp., 1 Neisseria cinerea, 1 Serratia plumutica, 1 Sphingomonas paucimobilis, 2 Streptococcus). In only one case, CNS was isolated in blood cultures of a patient. Of the contaminated products, 22 were thawed in a bath at 37° and 3 in a dry bath (p=0,02). There were no relevant technical problems during the washing of HSCs. During infusion, the following reactions were noted: desaturation (n=2), nausea and vomiting (n=8), and vasovagal syncope (n=5), although none of them were serious.
Conclusion: The semi-automatic washing HSCs is associated with a low percentage of infusional AE without causing significant cell loss or contamination of the product obtained, and this is even lower if the thawing is done in dry heat bath.

**P816**
**Characterisation of the different graft sources: a prospective series**
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Introduction: Outcomes and immune reconstitution in patients receiving an allograft may depend on distinct cellular components present in the donor graft.
Objective: To compare the cellular composition of several grafts samples (cryopreserved cord blood graft after thawing (CB, n=7) *vs* unmanipulated bone marrow graft (BM, n=5) *vs* G-CSF mobilised leukapheresis product (LP, n=10) by using a 6-color flow cytometric analysis (FACS CANTO II) and a very large panel of monoclonal antibodies. Proportions derived from comparison to total nucleated cells (TNC), lymphocytes or T cells. The two non-parametric tests of Kruskall-Wallis and Wilcoxon were used for statisical analyses.
Results: The median absolute number of NC was found significantly higher in LP (505.5 10*8, range: 265.2-836.6) compared to BM (125 10*8, range: 71.5-177) or CB graft (12.8 10*8, range: 3.6-20.1) (p<0.0001). The same median proportions of total T cells (BM 72% *vs* LP 61.5% *vs* CB 66%), CD4+/CD8+ T cells (BM 2.5% *vs* LP 0.95% *vs* CB 1.2%), TCR alpha/beta+ and TCR gamma/delta+ T cells (BM 97.5% and 2.5% *vs* LP 98.1% and 1.9% *vs* CB 98% *vs* 2%), total NK cells (BM 11% *vs* LP 18.05% *vs* CB 22.7%), NKT cells (BM 8.8% *vs* LP 7.65% *vs* CB 7%) were found between the three groups. The median proportion of mDCs was significantly higher compared to pDCs in CB graft only (0.2% *vs* 0.07%, p=0.01; BM 0.55% *vs* 0.19%, p=0.06; LP 0.26% *vs* 0.31%, p=0.55). All median proportions of the following cells components were found significantly higher respectively in 1) LP: monocytes (33.7% *vs* BM 3% *vs* CB 11.3%, p=0.0002), total lymphocytes (35.15% *vs* BM 13% *vs* CB 25.3%, p=0.004), memory T cells (49% *vs* BM 40% *vs* CB 9%, p=0.0007), B cells (5.84% *vs* BM 1.36% *vs* CB 3.37%, p=0.0002), Tregs (0.88% *vs* BM 0.23% *vs* CB 0.71%, p=0.003) and pDCs (0.31% *vs* BM 0.19% *vs* CB 0.07%, p=0.001); 2) CB graft: naïve T cells (91% *vs* BM 60% *vs* LP 51%, p=0.0007), CD4+/CD8- T cells (71.9% *vs* BM 51.3% *vs* LP 61.9%, p=0.003, pre-B cells (1.67% *vs* BM 1.38% *vs* LP 0.85%, p=0.01) and CD5+ B-cells (1.84% *vs* BM 0.41% *vs* LP 1.12%, p=0.002) and 3) BM: CD4-/CD8+ (41% *vs* LP 30% *vs* CB 22.5%, p= 0.005), CD4-/CD8- T cells (5.3% *vs* LP 3.35% *vs* CB 2.7%, p=0.04), mDCs (0.55% *vs* LP 0.26% *vs* CB 0.2%, p=0.04) and CD34+ stem cells (0.72% *vs* LP 0.48% *vs* CB 0.31%, p=0.01).
Conclusion: This original study shows strong differences in term of quantitative and qualitative cellular composition between several grafts samples, possibly explaining the differences observed in term of outcomes after the considered type of allograft.

**P817**
**Characterisation of the two different blood sources of graft (non-manipulated cord blood and G-CSF mobilised peripheral blood) as compared to peripheral blood: a prospective series**
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The majority of donor grafts arises currently from two different sources of blood samples: G-CSF mobilised peripheral blood (GCSF) and cord blood (CB).
Objective: To study the cellular composition of these two different blood sources of graft (CB n=10; GCSF n=10) as compared to peripheral blood (PB n=10) by using a 6-color flow cytometric analysis (FACS CANTO II) and a very large panel of monoclonal antibodies. Proportions derived from comparison to total nucleated cells (TNC), lymphocytes or T cells. The two non-parametric tests of Kruskall-Wallis and Wilcoxon were used for statisical analyses.
Results: The median absolute number of TNC was found significantly higher in GCSF (51205/µL) compared to CB (8495/µL) or PB (5525/µL) (p<0.0001). The same median proportions of monocytes (PB 7.25% *vs* GCSF 5.75% *vs* CB 9.05%), total T cells (PB 78.5% *vs* GCSF 64.95% *vs* CB 66%), TCR alpha/beta+ and gamma/delta+ T cells (PB 98% and 2% *vs* GCSF 97.5% and 2.5% *vs* CB 97.75% and 2.25%) and NK cells (PB 12.75% *vs* GCSF 17.85% *vs* CB 23.5%) were found between the three groups. The same median proportion of CD34+ stem cells was observed between GSCF and CB(0.12% *vs* 0.2%, p=0.45). All median proportions of the following cells components were found significantly higher in CB: naïve T cells (94.5% *vs* GCSF 50% *vs* PB 47.5%, p<0.0001), CD4+/CD8- T cells (74.25% *vs* GCS 65.75% *vs* PB 64.5%, p=0.007), CD4+/CD8+ T cells (1.55% *vs* GCSF 0.65% *vs* PB 0.95%, p=0.0009), pre-B cells (1.52% *vs* GCSF 0.14% *vs* PB 0.37%, p<0.0001), CD5+ B cells (0.99% *vs* GCSF 0.29% *vs* PB 0.55%, p=0.0005) and Tregs (0.92% *vs* GCSF 0.17% *vs* 0.54%, p<0.0001). The median proportions of the following cells components were found

significantly lower 1)in CB: memory T cells (5.5% *vs* GCSF 50% *vs* PB 52.5%, p<0.0001), CD4-CD8+ T cells (22.05% *vs* GCSF 29.9% *vs* PB 29.75%, p=0.04), CD4-/CD8- T cells (2% *vs* GCSF 4% *vs* PB 4.6%, p=0.004) and NKT cells (2.4% *vs* GCSF 9.2% *vs* PB 10.8%, p<0.001) 2)in GCSF: total lymphocytes (8.5% *vs* PB 30.7% *vs* CB 31%, p<0.0001), mDCs (0.05% *vs* PB 0.18% *vs* CB 0.42%, p=0.0001) and pDCs (0.07% *vs* PB 0.13% *vs* CB 0.11%, p=0.002). The median proportion of mDCs as compared to pDCs was significantly higher in CB only (0.42% *vs* 0.11%, p=0.002; GCSF 0.05% *vs* 0.07%, p=0.13; PB 0.18% *vs* 0.13%, p=0.09).
Conclusion: This original study shows strong differences in term of quantitative and qualitative cellular composition of two different blood sources of graft as compared to PB, directly influencing the final graft cellular composition used for the allotransplant.

**P818**

**Improvement in the outcome of allo-HSCT in the last 30 years: a single-centre experience**

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Objectives: Many clinical advances in the management of allohematopoietic stem cell transplantation (allo-HSCT) have been made. We analyze if these advances improve on the outcome of allo-HSCT in our center.
Methods: 712 allo-HSCT were done in the last 30 years in our hospital: 565 related and 147 unrelated. The source of progenitor cells was bone marrow in 577, peripheral blood in 97 and cord blood in 38. We divided patients according to the decade in which the allo-HSCT was done: 1980-1990 (n=166); 1991-2000 (n=248) y 2001-2010 (n=298).We analyzed the characteristis of donors and receptors, source of stem cells,conditionig regimen, aGVHD prophylaxis, aGVHD incidence,transplant related mortality (TRM), relapse and survival.
Results: The mean age of the receptor has increase: 20 y in the first, 28 y in the second and 31 y in the third decade (p<0,05). Bone marrow was the only source of stem cells in the first decade dropping to a 57% in the third decade. Unrelated allo-HSCTs were a 12,5% in the second decade and a 38,5 % in the third. Mortality has decreased significantly in each decade (59,6% in the first and 45% in the third; p<0,05). In related allo-HSCT we observe a reduction of mortality (50,4% in the second decade and 41,5% in the third), but this reduction is seems remarkably in unrelated allo-HSCT with a mortality of 71% in the second decade and 50,4% (p=0,041) in the third decade. In idintical-sibling allo-HSCT, the MRT-100 has dropped from 10,6% in the first decade to 7,2% in the third (p<0,05). Mortality due to relapse has fallen (30,6% in the first decade and 17,9% in the third; p<0,05) as well as mortality due to aGVHD (12,8% in the first decade *vs* 6% in the third; p<0,05.The appearance of aGVHD grade II-IV *vs* grade 0-I was associated with an increase in mortality in receptors with non-malignant diseases (42,8% *vs* 14,4%; p=0,023) but in receptors with malignant diseases was lesser (35,9% *vs* 41,4%; p=0,02).
Conclusions: Despite an increase in the mean age of the receptors and an increase in the complexity of allo-HSCT we observe a significant reduction in the mortality in the last decade as well as a significant reduction in the TRM-100 and in the mortality due to relapse (probably due to an indication of the transplantation in early phases of the diseases). We observe a significant reduction of relapse in receptors with aGVHD II-IV and a significant improvement in the survival of receptors that receive an unrelated allo-HSCT in the last decade.

**P819**

**Highly significant impact of HLA-DRB3 and -DRB4 matching on different unrelated allo-HSCT outcomes: new perspectives in the unrelated donor selection**

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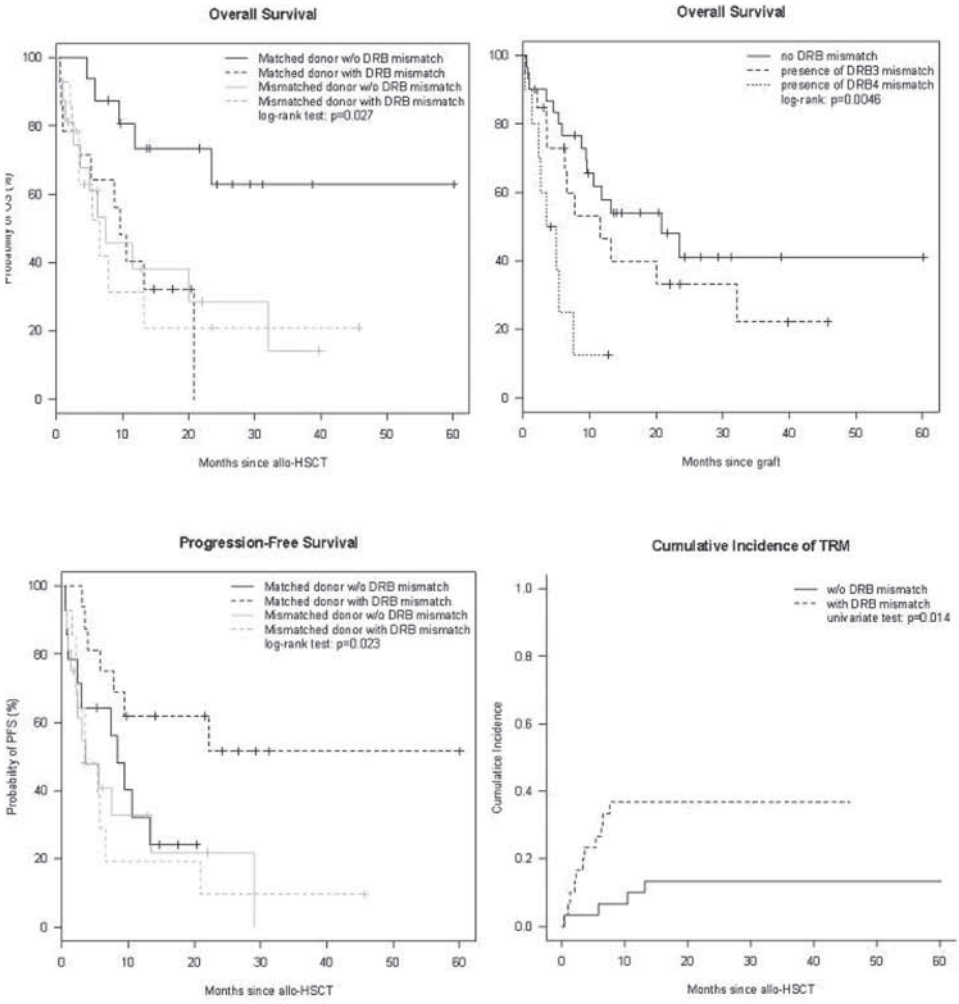
We examined retrospectively in our center the outcome of 30 patients who received unrelated HSCT with a HLA-DRB3 or -DRB4 mismatched donor (study group) and we compared with a cohort of 30 patients (control group) with the same characteristics except for the DRB3 or DRB4 donor mismatching. In the study group, there were 16 patients with 10/10 HLA identical donor among them 11 had a DRB3 mismatch and 5 had a DRB4 mismatch. There were 14 patients with 9/10 HLA identical donor among them 9 had a DRB3 mismatch and 5 had a DRB4 mismatch. In the control group, there were 16 patients with 10/10 HLA identical donor and 14 patients with 9/10 HLA identical donor. After HSCT, 27 (90%) patients in the study group engrafted while 29 (96%) engrafted in the control group. The cumulative incidence of acute GVHD≥2 at 3 months was 37% (95%CI, 28-46) and 30% (95%CI, 22-39) for the study and control groups respectively, with a same cumulative incidence of chronic GVHD at one year of 20% (95%CI, 12-28). At day 90 post HSCT, 17 (63%) patients in the study group were in CR and 25 (86%) in CR in the control group. After a median follow-up of 5 months (range, 0.2-46) and 13 months (range, 0.5-60) for study and control groups respectively, the median OS was 7 months (range, 3-32) and 21 months (range, 11-NR) with a 2-years probability of 25% (95%CI, 12-51) and 41% (95%CI, 25-69) respectively; the median PFS was 3 months (range, 0.2-46) and 10 months (range, 1-60) with a 2-years probability of 16% (95%CI, 6-42) and 37% (95%CI, 21-63) respectively. The cumulative incidence of relapse at 1 year was the same for the two groups with 30% (95%CI, 22-39); the cumulative incidence of TRM at 3 months and 1 year were 17% (95%CI, 10-24) *vs*. 3% (95%CI, 0-7) and 37% (95%CI, 28-46) *vs*. 10% (95%CI, 5-16) for study and control groups respectively. The multivariate analysis showed a significant worse OS in 9/10 mismatched patients with or without a DRB3 or DRB4 mismatch (HR=5.3; [95%CI, 1.6-18] p=0.006); 10/10 matched patients with a DRB3 or DRB4 mismatch (HR=3.9; [95%CI, 1.2-12] p=0.02) and patients not in CR at transplantation (HR= 4.4; [95%CI, 1.6-12] p=0.004); similarly, the same groups had a worse TRM in multivariate analysis, (HR=6; [95%CI, 1.5-24] p=0.02) and (HR=3.5; [95%CI, 1.02-12] p=0.04) respectively. In view of the important impact of these loci mismatches on clinical outcomes, it should be considered in the unrelated donor selection.

**[P819]**




**P820**

**Use of haploidentical stem-cell transplantation with KIR-ligand mismatch as salvage therapy for poor-risk acute lymphoblastic leukaemia**

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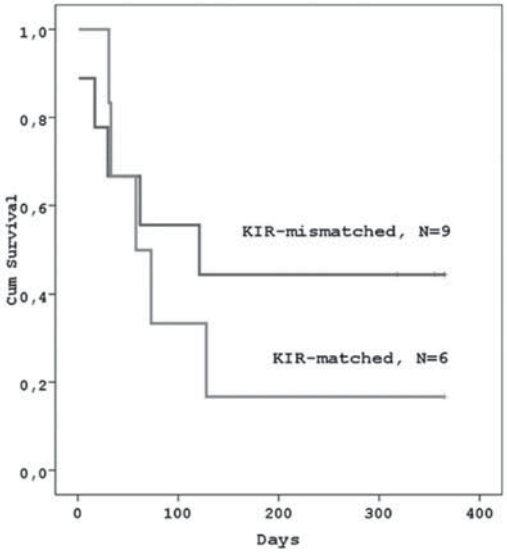
Introduction: Haploidentical hematopoietic stem cell transplantation (haplo-HSCT) has made tremendous progress over the past 20 years and has become a feasible option for leukemia patients without HLA-matched related or unrelated donors. The early complications including severe acute graft-versus-host disease (aGVHD), graft failure, delayed engraftment and in addition, disease recurrence limited the usage of this approach. NK-cell alloreactivity is determined by the presence in the donor of NK cells expressing inhibitory killer cell Ig-like receptors (KIRs) that recognize HLA class I allotypes present in the donor but lacking in the recipient. This phenomenon can improve event-free survival (EFS) after haplo-HSCT, especially in AML patients.

Aim: To compare efficacy of KIR-ligand mismatched and matched haplo-HSCT in patients with very high risk of acute lymphoblastic leukemia (ALL).

Methods: Fifteen children and adolescents with ALL were included in the study. Median age 8 y.o. (1-19), sex ratio F:M 9:5. The median number of infused CD34+ cells was 10,2×106 (range 4,5-25,8) per kg b.w. Children with evidence of donor engraftment who survived more than 14 days and more than 90 days from transplantation were evaluated for occurrence of aGVHD and chronic GVHD, respectively, according to established criteria. aGvHD I-II0 – 4pts, aGvHD III-IV0 – 3pts; chGvHD limited – 2pts, chGvHD extensive – 3pts.

Results: One-year overall survival (OS) was higher in patients after KIR-ligand mismatched haplo-HSCT compared with KIR-ligand matched haplo-HSCT: 43% and 18% respectively. aGVHD was investigated in 7 pts, but it was not the main reason of mortality (III-IV stage in 3 pts). Five patients alive and achieved complete remission. Conclusions: Haplo-HSCT is effective therapy for patients with very high risk acute leukemia. Apparently, NK- alloreactivity increased OS in patient with very high risk ALL. The main reason of treatment failure after haplo-HSCT was relapse which requires continuing therapy by immunotherapy with/without chemotherapy.

**[P820]** **OS after Haplo-HSCT in ALL patients KIR-ligand matched vs KIR-ligand mismatched**




**P821**

**Unmanipulated HLA-mismatched/haploidentical peripheral blood stem cell transplantation for high-risk haematologic malignancies**

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Objectives: Haploidentical hematopoietic stem cell transplantation (HSCT) has been increasingly applied in high-risk hematologic patients due to the absence of HLA matched donors. The aim of this study is to investigate the efficacy and safety of unmanipulated haploidentical allogeneic peripheral blood stem cells transplantation (PBSCT) for hematologic malignancies.
Study Design and Methods: Patients who underwent unmanipulated HLA-mismatched/haploidentical PBSCT from July 2007 to March 2010 with high-risk hematologic malignancies were enrolled for retrospective analysis.
Results: Twenty-one patients with high-risk hematologic malignancies underwent unmanipulated HLA-mismatched/haploidentical PBSCT with myeloablative conditioning. The numbers of CD34+ cells infused at transplantation were 4.81 (2.61-11.47) ×106/kg. Patients achieved myeloid and platelet engraftment at a median of 16.5 days and 20 days, respectively. The cumulative incidence of acute graft-versus-host disease (aGVHD) on day 100 was 52.7±10.7%, and the 2-year cumulative incidence of chronic graft-versus-host disease (cGVHD) was 39.5±10.6%. Cumulative incidence of CMV antigenemia and hemorrhagic cystitis within 100 days after PBSCT were 59.5±16.7% and 34.8±13.3%, respectively. One hundred-day transplantation related mortality (TRM) rate and the 2-year cumulative TRM rate were 14.3% and 20.5±7.8%, respectively. The 2-year cumulative overall survival was 62.1±11.4% and the probability of disease-free survival at 2-years was 55.6±10.7% with a 16-months median follow-up.
Conclusion: Unmanipulated PBSCT is a promising protocol in HLA-mismatched/haploidentical transplant settings.

**P822**

**Early engraftment, full donor chimerism and low toxicity after myeloablative cord blood plus haploidentical donor dual transplantation in older patients with high-risk myeloid leukaemia**

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Introduction: The umbilical cord blood (UCB) plus haploidentical donor dual transplant strategy pioneered by Fernández *et al* (Madrid, Spain), showed improved UCB transplant outcomes by hastening hematopoietic recovery. Recently, Liu *et al* have used modified a reduced intensity protocol in an older cohort of patients, reproducing the early engraftment kinetics, but also leading to persistent mixed chimerism and raising concerns about the risk of relapse in this setting. Here, we present our own experience with a non-TBI myeloablative dual transplant strategy in older patients with high-risk AML.
Methods: Eight consecutive high-risk MDS/AML patients (age 50 years, 32-64; 5 refractory to >1 chemotherapy lines, 2 relapsed after previous BMT, 2 with active AML at time of BMT (17% and 22% blasts); 02/2009-07/2011; Table 1), received 12 mg/kg Busulfan, 100 mg/kg Cyclophosphamide, 120 mg/m$^2$ Fludarabine, and 4 mg/kg Thymoglobulin, followed by UCB single units (2.84x10$^7$/kg median TNC and 1.16x10$^5$/kg CD34+ cells), and haploidentical mobilized CD34+ cells (median 4.72x10$^6$/kg, with <1x10$^4$/Kg CD3+). GVHD prophylaxis included cyclosporine and prednisone.
Results: All cases engrafted neutrophils (>0.5x10$^9$/L; day +11, 10-17) and platelets (>20 x10$^9$/L; +21, 12-95), and there were no secondary graft failures. Two cases had grade II acute GVHD, and 1 case had a quiescent chronic limited GVHD. Opportunistic infections occurred mainly after granulocyte engraftment: 3 CMV reactivations (2 in the context of treatment for of acute GHVD) and 2 hemorrhagic cystitis by BK virus.

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Table 1. Patient demographics, characteristics of the cellular products and follow up.

| | Sex | Age | Wt | Primary Disease | Cord Blood Unit | | | Haploidentical Donor | | | Follow Up | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | HLA matching | TNC x10^7/kg | CD34 x10^5/kg | Donor | CD34 x10^6/kg | CD3 x10^4/kg | Months | Status |
| 1 | M | 53 | 62 | AML secondary to MDS | 5 / 6 | 3,71 | 1,5 | Sister | 4,46 | 0,15 | 34 | Alive - CR |
| 2 | F | 52 | 95 | AML/RAEBt (22% blasts) | 4 / 6 | 2,05 | 1,8 | Daughter | 11,90 | 0,80 | 26 | Alive - CR |
| 3 | F | 64 | 66 | AML Flt3-ITD | 4 / 6 | 2,62 | 0,84 | Daughter | 3,68 | 0,15 | 7 | Dead in CR |
| 4 | M | 62 | 68 | RAEB-II (17% blasts) | 5 / 6 | 1,96 | 0,98 | Daughter | 4,47 | 0,20 | 16 | Alive - CR |
| 5 | F | 37 | 71 | AML Flt3-ITD | 4 / 6 | 5,8 | 1,68 | Sister | 4,96 | 0,40 | 16 | Alive - CR |
| 6 | M | 48 | 58 | AML | 4 / 6 | 3,43 | 0,81 | Sister | 5,89 | 0,30 | 14 | Alive - CR |
| 7 | F | 32 | 99 | AML Flt3-ITD, trisomy 5 | 4 / 6 | 2,31 | 0,35 | Brother | 3,62 | 0,40 | 8 | Alive - CR |
| 8 | F | 40 | 56 | AML | 4 / 6 | 3,05 | 1,34 | Brother | 5,99 | 0,73 | 5 | Alive - CR |

AML: acute myeloid leukemia; F: female; ITD: internal tandem duplication; M: male; MDS: myelodysplastic syndrome; RAEB: refractory anemia with excess blasts; RAEBt: RAEB in transformation (20-30% blasts); TNC: total nucleated cells; Wt: weight fin kg)

Table 2. Kinetics of donor chimerism.

| | Chimerism Analysis (CBU / Haplo / Host)§ | | | | |
|---|---|---|---|---|---|
| | 2 weeks | 1 month | 3 months | 6 months | 12 months |
| 1 | 33% / 67% / – | >95% / <5% / – | 100% / – / – | 100% / – / – | 100% / – / – |
| 2 | 12% / 63% / 25% | 62% / 24% / 14% | 100% / – / – | 100% / – / – | 100% / – / – |
| 3 | 31% / 69% / – | >95% / <5% / – | 100% / – / – | 100% / – / – | n/a |
| 4 | 14% / 86% / – | n/a | 50% / 50% / – | 90% / 10% / – | 100% / – / – |
| 5 | – / 100% / – | <5% / >95% / – | 53% / 47% / – | 100% / – / – | 100% / – / – |
| 6 | 6% / 94% / – | 75% / 25% / – | 100% / – / – | 100% / – / – | 100% / – / – |
| 7 | 10% / 90% / – | 75% / 23% / <5% | 49% / 46% / 5% | 95% / 5% / – | n/a |
| 8 | 8% / 92% / – | 49% / 51% / – | 100% / – / – | n/a | n/a |

§: percentage of chimerism in peripheral blood coming from CBU, Haploidentical graft and Host.

Patients became full donor chimeras at 3-6 months after transplant, shifting from haploidentical donor to full CBU chimerism in whole peripheral blood, lymphoid and myeloid subsets, and in the bone marrow (Table 2). There was one non-relapse death 7 months after transplantation; all other patients remain alive, in CR and full UCB chimerism at last follow-up (median 15 months, 5-34), including both patients with active disease, now 26 and 14 months after transplant.

Conclusions: These data reproduce the promising results of the UCB-haploidentical strategy in an independent series of patients. They also suggest that shorter time to engraftment may be a main driver of improved outcome in this strategy and confirm that full CB chimerism can be rapidly achieved following a non-TBI myeloablative conditioning in an older cohort of high-risk patients with good control of the underlying high-risk AML.

**P823**

**Treatment of relapses, graft rejection or absence of engraftment after first allogeneic stem cell transplantation: a single-centre experience**

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Introduction: Despite of well known efficacy of allogeneic stem cell transplantation (SCT) for the treatment of different hematological malignancies, relapses are still possible and till now there is no standard approach neither for relapses, nor for other SCT failures. Current options for treatment of relapses after primary SCT are chemotherapy, donor lymphocyte infusion or second SCT.

Aim: To estimate efficacy of second allogenic SCT in the treatment of relapses, graft failure or absence of engraftment after primary SCT.

Patients and Method: From 1995. till 2010., 18 patients (pts) undergone second SCT for treatment of relapse (13 pts), graft rejection (2 pts) or absence of engraftment (3 pts) after first SCT. Median age in this cohort of pts was 23 (16-32) years, M/F ratio 11/7. Pts were suffering from various diseases: 2 AML, 9 ALL, 4 CML, 1 MDS, 2 AA. Median follow up is 35 (range 11-192) months. Relapse had occured at a median of 19,1 months after first allo SCT (range 7-73). Graft rejection was observed after one year from first SCT in both cases with aplastic anaemia. Pts with acute and chronic leukemias had received "salvage" chemotherapy (Flag-IDA) and afterwards despite of marrow findings, underwent second allogeneic SCT with reduced intensity conditioning. Pts with aplastic anaemia were conditioned with Cyclophosphamide and ATG. All pts had received stem cells from same identical sibling donor. Peripheral blood was source of stem cells in 16 pts and "primed" bone marrow in 2 pts. Prevention of graft versus host disease (GvHD) was modified according to specific situation (complete absence of prophylaxis in the cases of leukemia relapses or combination of Cyclosporin A with Metothrexate or MMF in the graft rejection or graft failure).
Results: Engraftment was observed in all pts with respect to count of polymorphonuclears after 16 (11-23) days and platlets after 21 (12-37) days. Acute (a) GvHD was observed in 6 pts (33,3%) and chronic (c) GvHD in 12 pts (66,6%). Overall survival (OS) of all our pts is 33,3% with median follow up 35 (11-192) months. Vast majority of alive pts had mild or moderate cGvHD without serious influence on their quality of life.
Conclusion: Our modest results have showed benefit of second SCT as treatment option for selected cohort of pts who have failed after first allografting. Further investigation on larger, homogenuos groups of pts is needed.

**P824**
**Transplantation of haematopoietic stem cells in human severe combined immunodeficiency: experience of a single centre**
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Objective: To analyze outcomes of 15 patients with severe combined immunodeficiency (SCID) treated by hematopoietic stem cell transplantation (HSCT) from haploidentical donors (HD), unrelated cord blood donors (UCBD) and human leukocyte antigen (HLA) unrelated donor (URD), at six months from transplant.
Methods: A retrospective study of medical records from 15 patients transplanted for SCID who received HSCT between 2001 and 2011 at our center (Hospital Universitario La Paz, Madrid, Spain).
Results: 15 patients with SCID were treated with HSCT, 7 received HD from peripheral blood (group A), 6 UCSCD (group B) and 2 URD from bone marrow (group C). Median age at transplant was 17.2 months. The molecular defects were known in 66% of our patients, being the most frequent those who have mutations in the gene encoding the common gamma chain, RAG1/RAG2 and HLA class II deficiency (Table 1). Survival rate at six months was 71% in group B, 100% in group C and 50% in group A. Incidence of grade II-IV acute graft-versus-host-disease (GVHD) were 16% in group A, 33% in group B and 50% in group C. About post transplant infections, only four patients (26.6% of the total) from group A did not develop infection by gram negative. Cytomegalovirus pneumonitis after HSCT was observed in 28.5% of patients from group A and 33.3% in group B. Chimerism was evaluated at six month, 43% of patients in group A presented complete chimera (CC), 67% in group B and 50% in group C.
Conclusions: Our study suggests that other sources of hematopietic stem cells as haploidentical and cord blood donors should be consider in the absence of a relative with identical HLA.

**P825**
**Inactivated human platelet lysate is a new method to ensure safer GMP-compliant MSC production**
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Introduction: Mesenchymal Stem Cells (MSCs) are ideal candidates in regenerative and immunomodulatory therapies. The use of xenogenic protein free GMP-compliant growth media, is a mandatory prerequisite for clinical-grade MSC isolation and expansion. Pooled Human Platelet Lysate (HPL) has been efficiently implemented into MSC clinical scale manufacturing as an animal serum substitute. In this study, we compared HPL as a supplement of MSC culture medium to Foetal Bovine Serum (FBS) usually used for MSC expansion. Moreover, to upgrade quality/safety we decided to test inactivated HPL (iHPL) compared to non-inactivated HPL for use in the clinical-scale expansion of MSCs.
Methods: BM samples were directly plated at a density of 10000 cells/cm$^2$ using 3 different culture mediums: 1) alphaMEM+10 % HPL; 2) alpha MEM+10% HPL (iHPL ) both with+ 2U/ml of heparin; 3) MSC medium (Stem Cell Technologies) containing 10% foetal bovine serum (FBS), usually used for MSC isolation and expansion. HPL was obtained from 10 to 15 Buffy-Coats derived platelet concentrates (BC-PCs) and iHPL was inactivated with INTERCEPT Blood System (Cerus).
At each passage, MSC morphology, cellular growth (cPD), immunophenotype (CD90, CD73, CD105, 45-34-14, CD271 and CD146), kariotype and sterility were analyzed. Statistical analyses were performed with Wilcoxon signed Rank Test-paired samples.
Results: MSCs cultivated in alphaMEM+10% HPL (HPL group) appeared smaller and more numerous than MSCs in MSC medium (MSC medium group). Between the two groups, no differences were observed in the number of fibroblast colony-forming cells (CFU-F), but the CFU-F of HPL group are richer in the number of cells than those of MSC medium group. At the 3rd passage, the cPD of MSCs was 10% higher in the HPL cell group than in the MSC medium group, while no differences were observed in terms of immunophenotype, multipotent capacity or kariotype. The sterility was negative in all the samples analyzed. No statistical differences were found among iHPL and HPL in terms of MSC expansion efficiency.
Conclusion: HPL represents a good GMP-compliant alternative to animal serum for MSC clinical production and is more advantageous especially in terms of cellular growth. Moreover, the inactivation procedure improves HPL quality in terms of safety.

## Stem Cell Donor

**P826**
**Interleukin-10-592 CC genotype in the donor confers strong protection against relapse after allogeneic haematopoietic stem cell transplantation in children with haematological malignancies**
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Interleukin-10 (IL-10) is a pivotal immunomodulatory cytokine, which can promote the growth of activated CD8+ T cells leading to the maintenance of antitumor function *in vivo*. The IL10-592 CC genotype is associated with higher production of IL-10 than the AC or AA genotype. Because IL-10 may promote the development of alloimmunity we hypothesized that the

[P824] Table1. Diagnosis, complications and outcomes of patients with SCID

| Patient No. | Phenotype / Molecular defect | HSCT source | Age (in months) | Infections | AGvHD | Chimerism | Outcome |
|---|---|---|---|---|---|---|---|
| 1 | Other | HD | 11 | Gram - / CMV pneumonitis | Yes | CC | Alive |
| 2 | T-B-NK+, RAG1/RAG2 | UCBD | 8 | Gram - / CMV pneumonitis | No | PC | Alive |
| 3 | T-B+NK-, γc | UCBD | 2 | Gram - | No | CC | Alive |
| 4 | T-B+ | UCBD | 7 | Gram - | No | CC | Alive |
| 5 | T-B+NK-, γc | UCBD | 9 | Gram - | Yes | CC | Alive |
| 6 | T-B+ | HD | 8 | CMV pneumonitis | No | NQ | Deceased |
| 7 | CD4-, CD8+, B+NK+, HLA II | UCBD | 19 | Gram - | Yes | CC | Alive |
| 8 | T-B+ | HD | 23 | Gram - | No | PC | Alive |
| 9 | T-B+NK-, JAK3 | URD | 9 | Gram - | Yes | CC | Alive |
| 10 | T-B+ | HD | 6 | CMV / SRV | No | NQ | Deceased |
| 11 | CD4-, CD8+, B+NK+, HLA II | HD | 44 | Gram - | No | NQ | Deceased |
| 12 | T-B-NK+, RAG1/RAG2 | URD | 17 | Gram - | No | NQ | Deceased |
| 13 | Omenn syndrome | UCBD | 7 | No | Yes | NQ | Alive |
| 14 | T-B- | HD | 11 | Gram - / CMV | No | CC | Deceased |
| 15 | T-B-NK- | HD | 78 | Pulmonary aspergillosis | No | CC | Alive |

Abbreviations: HSCT, hematopoietic stem cell transplantation; HD, haploidentical donor; UCBD, unrelated cord blood donors; URD, unrelated donor; Gram -, gram negative; CMV, cytomegalovirus; RSV, respiratory syncytial virus, AGvHD, acute graft-versus-host-disease; CC, complete chimera; PC, partial chimera; NQ, non chimera

IL-10-592 CC genotype in the donor reduces the risk of relapse after hematopoietic stem cell transplantation (HSCT). A cohort of 211 children (median age, 12 years) with acute lymphoblastic leukemia (n=100), acute myeloid leukemia (n=62), myelodysplastic syndrome (n=30) or chronic myeloid leukemia (n=19) who underwent allogeneic bone marrow (n=153) or peripheral blood stem cell transplantation (n=58; T-cell depleted: n=26) in a single center and/or their respective donors was genotyped of IL-10 gene for rs1800872 using TaqMan real-time polymerase chain reaction. The donor was HLA-matched unrelated in 48%

of transplants and HLA-identical related in 42% of transplants. Conditioning regimen was myeloablative in all cases. Two forms of post-transplant immunosuppression predominated, cyclosporine A and methotrexate in 69% of transplants and cyclosporine A alone in 17% of transplants. Cell samples from the donor were available in 174 cases and from the patient in 197 cases. The IL-10-592 CC genotype was present in 82 of the 174 donors (47.1%) and in 104 of the 197 patients (52.8%). Interestingly, we found a significantly reduced incidence of relapse in patients who were transplanted from a donor with the IL-10-592 CC genotype (15.9% versus 30.4%; p=0.016). In addition, we observed a significant increase of event-free survival (p=0.019) and a significant increase of overall survival (p=0.040) if the IL-10-592 CC genotype was present in the donor. The occurrence of the IL10-592 CC genotype, in either donors or recipients, had no significant impact on treatment related mortality, acute and chronic graft-versus host disease. In conclusion, IL-10-592 CC genotype in the donor confers strong protection against relapse leading to a significant increase of event-free survival and overall survival after HSCT in children with hematological malignancies. This is the first study to describe an association of IL-10 gene polymorphism with relapse rate after HSCT. Selecting a donor with the IL-10-592 CC genotype could be a useful therapeutic strategy for improving the final outcome after allogeneic HSCT.

**P827**

**Lenograstim-mobilised haematopoietic progenitor cells in related healthy donors older than age 55 years: efficacy, short- and long-term side effects compared with younger donors**

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Background: G-CSF-mobilized Hematopoietic progenitor cells (HPC) and collected by apheresis as a source of stem cells, are increasingly employed for allogeneic transplant. Each country has one or more national HPC donor registries and, in Italy, the unrelated healthy donors (HDs) have to sign the initial consent acknowledging that they have been informed about registration on a donor list, giving their availability to be called until their 55th birthday.
Aim: To evaluate whether Lenograstim-mobilized HPC in "elderly" HDs (i.e. aged more than 55 years at the day of apheresis) is safe and effective as in young HDs (i.e. younger than 55 years at the day of apheresis). Material and Methods: 231 consecutive, non selected related HDs were compared: young HDs (n=185, mean age 40.6 years, range 15-54.8) and elderly HDs (n=46, mean age 59.6, range 55.1-70.0 years).
Results: The two groups were comparable in terms of gender, body-weight, G-CSF dose and days of administration. CD34+ cell blood peak value (cells/microl) was higher in the young HDs group on day 4 (48±35 *vs* 34±26 R=-0-16 p=0.01) but not on day 5 (72±50 *vs* 72±107, R=.0.02 p=0.7). The WBC peak (x$10^9$/L) was higher on day 4th and 5th in the young HDs group (42±13 *vs* 36±8, p=0.001, and 43±13 *vs* 39±11, p=0.014, respectively). The G-CSF-related side effects were similar in the two groups with the exception of bone pain (moderate to severe) that was more frequent in young HDs (40% *vs* 22%, p= 0.015. Aphaeretic collections were started on day 5th and the volume of processed blood normalized for blood volume were the same in both groups (respectively 2.7±0.7 and 2.4±0.6 p= ns). A CD34+ cell dose >4x10E/kg/recipient was reached at the 1st apheresis in a similar percentage of HDs (60% in young *vs* 54 % in old HDs group, p=ns). 5 HDs in young group and 1 HD in old group failed mobilization, with a CD34 dose <2x10E6/kg recipient. After a follow up of 87±45 months in young HDs and 80±49 in old HDs (p=ns) no haematological malignancies were observed in both groups.
Conclusion: Short-term safety and efficacy are equivalent between the two groups. The study did not identify any increase in the risk of malignancy among old HDs who received G-CSF. The limit of 55 years in national registers for HPC donation should be reconsidered.

**P828**

**Hypokalaemia in healthy donors who underwent apheresis of peripheral blood stem cells: a case of paroxysmal atrial fibrillation**

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Introduction: The apheresis of peripheral blood stem cells (aPBSC) is generally safe but not completely risk-free. The adverse events in PBSC healthy donors (HD) were reported in 0.6% of cases, the great majority of which were represented by hypocalcemia, vasovagal reactions, thrombocytopenia and problems with vascular access. Hypokalemia has not been sufficiently considered. The pathogenesis of hypokalemia during a PBSC collection is unknown and it may be due to the metabolic processing of the dextrose that stimulates the release of insulin and cause intracellular shifting of potassium. Patients. Between 2006 and 2011, 45 HD were subjected to 88 aPBSC. The mean age of HD was 47.6 years. The HD have been subject to assessments in accordance with the standards GITMO for PBSC donors. G-CSF was administered at a dose of 10 µg/Kg/day s.c. On average 7.94 (±2.22) x 10^6 CD34+/Kg were collected processing average 11.8 L (2-18 L) of blood (on average 2.3 blood volume). The mean ACD-A infused was 1.08 L (±0.281 L). On average were administered 3 fl of calcium(Ca)-gluconate for aPBSC: 40 HD received Ca-gluconate in continuous infusion and 3 HD at the onset of paresthesias. Results. In 72.4% of HD was observed a significant reduction in the levels of potassium (K+) after aPBSC: the mean before aPBSC was 4.09mEq/L and after aPBSC was 3.1 mEq/L (p<0.0001). In 42 HD (93.3%), hypokalemia was corrected with oral K+ supplementation, while in 3 HD (6.7%) the therapy was performed i.v. with 1-2 fl KCl. One female donor of 50-year-old developed asymptomatic and moderate hypokalemia (2.9 mEq/L) after aPBSC (the baseline K+ was 3.8 mEq/L). Despite the correction of K+ orally, about 10 hours after the end of aPBSC, the donor showed an episode of micturition syncope associated with paroxysmal atrial fibrillation (PAF). Conclusion. The problem of hypokalemia is not negligible, in our study involving up to more than 70% of HD undergoing aPBSC. K+ supplementation and dosing are largely empirical and guided by serum K+ levels. I.v. K+ supplementation is reserved for the treatment of severe or symptomatic hypokalemia. In one case of asymptomatic and moderate hypokalemia the correction of K+ orally has not been sufficient to prevent the onset of PAF. Perhaps the rapid development of hypokalemia after aPBSC would have required a treatment with i.v. K+ in order to correct more quikkly hypokalemia, to cancel the risk of arrhythmia and to preserve the physical integrity of the HD.

**P829**

**Single-centre experience with three different apheresis systems for autologous and allogeneic stem cell collections**

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Background: Peripheral blood progenitor cells (PBPC) are the most common stem cell source for autologous or allogeneic transplantation. Since 2008, three different collection systems were used in our hospital. Fresenius COM.TEC (Fresenius Healthcare, Bad Homburg, Germany) works with continuous

blood flow by collecting PBPC cyclically at the end of each individually determined separation cycle in a one stage separation chamber.
Both COBE Spectra and Optia (Caridian BCT, Garching, Germany) work with continuous blood flow too. While the COBE Spectra MNC apheresis system permits manually controlled continuous PBPC harvesting, the Spectra Optia provides an automated buffy coat interface control and combines centrifugation with subsequent cellular collection into an elutriation chamber with intermittent PBPC harvesting.
Methods: Between 2008 and 2011, in a single-center trial, 119 autologous and 53 allogenic apheresis procedures were compared to validate feasibility and effectiveness of the different systems.
Results: All three groups were comparable with regard to donor characteristics and the quality of the final product. In contrast, collection efficacy (CE1=[CD34+ cells collected]/[mean of CD34+ cells processed]) varied significantly with lowest results obtained with the Spectra. CE1 for COM.TEC and Optia was comparable; both instruments also produced lower white blood cell contamination in the product compared to the Spectra, but at the expenses of a higher product volume and longer apheresis time. The highest volume was obtained with COM.TEC. There was no significant difference in platelet loss between all three systems; however, a subgroup of allogeneic donors with high platelet counts lost more platelets with COM.TEC than with the other systems. Detailed results (mean and range) are given in Table 1.
Conclusions: Replacing COBE Spectra by Spectra Optia leads to significantly higher collection efficacy but longer procedure time which may be improvable with extended experience. Both Optia and COM.TEC have comparable collection efficacy. A lower product volume and less platelet loss in allogeneic donors may be favourable characteristics of the Optia system.

**P830**

**Best harvest for a successful bone marrow allograft and donor security**

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Equivalence between requested and collected product is mainly important. The recipient needs enough hematopoietic stem cells for a successful allograft but there is no need to collect a higher quantity of cells than required, which would imply for bone marrow harvest a longer time of anaesthesia, a higher volume harvested and a higher quantity of cells collected, consequently a longer time of recuperation.
We try to discriminate the elements which can explain this difference between requested and collected quantities in the aim of improving the process.
Material and Methods: Between January 2009 and November 2011, 2086 graft products were collected for French patients. Among them, 1472 are peripheral blood stem cells and 614 bone marrow harvests. For each couple donor/patient, the difference (higher or lower) between requested and collected product is noted, both in the case of bone marrow and in the case of peripheral blood stem cells. The proportional weight between donor and recipient related to the TNC/kg donor requested in bone marrow harvest, and the CD34/kg donor requested in PBSC collection are both specifically studied.
Discussion: For each couple donor/patient, a difference superior to 20% between requested and collected product is noted in about 70% of the products. Over a 20% difference, 50% of products collected are higher than requested, and 17% are lower.
If we consider Bone Marrow harvest, 25% are 20% lower than expected and 32% higher. The proportional weight between donor and recipient is not related to the success of bone marrow collection, though the rule is to stop bone marrow collection

**[P829]**

| Data Summary | COM.TEC | | COBE Spectra | | Spectra Optia | |
|---|---|---|---|---|---|---|
| allogenic | n=28 | range | n=14 | range | n=11 | range |
| age | 43 | 24-68 | 45 | 21-62 | 58 | 47-65 |
| WBCx10⁶/µl | 60 | 40-113 | 55 | 39-69 | 53 | 32-69 |
| CD34/µl | 90 | 30-154 | 101 | 37-244 | 95 | 45-166 |
| PLTx10³/µl | 200 | 80-390 | 225 | 159-317 | 208 | 149-239 |
| Flow rate (ml/min) | 50 | 45-55 | 66 | 40-90 | 53 | 42-68 |
| procedure time | 268 | 226-318 | 236 | 210-300 | 287 | 239-337 |
| TPV (ml) | 11824 | 9319-17831 | 15126 | 9121-23763 | 12583 | 9377-16205 |
| WBC in product | 312 | 145-458 | 375 | 229-517 | 233 | 204-272 |
| PV (ml) | 491 | 290-617 | 224 | 192-277 | 318 | 180-430 |
| CD34+x10⁶/kgKG | 6,4 | 1,73-14,57 | 7,42 | 2,27-15,7 | 7,26 | 3,27-10,4 |
| CE1 % | 65 | 41-91 | 36 | 22-47 | 49,9 | 29-61 |
| PLT loss % | 59,03 | 44,1-87,3 | 47 | 27-70 | 40 | 37-45 |
| CR (ml/kg) | 71,00 | 41-118 | 74 | 37-112 | 67 | 41-152 |
| CR/time | 0,26 | 0,14-0,43 | 0,32 | 0,14-0,5 | 0,3 | 0,18-0,45 |
| autologous | n=49 | | n=44 | | n=26 | |
| age | 38 | 20-69 | 57 | 26-71 | 60 | 23-69 |
| WBCx10⁶/µl | 31 | 7-113 | 39 | 5-69 | 36,4 | 9-99 |
| CD34/µl | 221 | 19-1490 | 209 | 25-1421 | 244 | 31-1163 |
| PLTx10³/µl | 86 | 13-279 | 112 | 22-459 | 103 | 34-250 |
| Flow rate (ml/min) | 51 | 45-55 | 67 | 48-86 | 58 | 38-75 |
| procedure time | 232 | 136-304 | 214 | 110-284 | 254 | 145-329 |
| TPV (ml) | 10463 | 6019-14278 | 13631 | 6501-20001 | 12046 | 6114-17212 |
| WBC in product | 210 | 64-324 | 316 | 72-992 | 235 | 151-294 |
| PV (ml) | 384 | 209-590 | 201 | 140-269 | 281 | 126-468 |
| CD34+x10⁶/kgKG | 10,96 | 1,11-42,6 | 11,75 | 1,9-51,2 | 15,1 | 2,6-44,4 |
| CE1 % | 61,61 | 36-83 | 49 | 9-82 | 68 | 41-113 |
| PLT loss % | 32 | 3-61 | 33 | 7-55 | 39 | 6-55 |
| CR (ml/kg) | 65 | 26-112 | 69 | 30-129 | 85 | 26-254 |
| CR/time | 0,28 | 0,15-0,55 | 0,32 | 0,17-0,48 | 0,3 | 0,1-1,0 |

at a volume of 20ml/kg donor. We observe that a low TNC collection is usually related to a low TNC concentration. When the collection is quite higher than expected, we suppose that the donor centre has no blood cell count available to estimate rightness of the collection. If we consider Peripheral Blood Stem Cell collection, 12% are lower than expected, and 59% higher. The proportional weight between donor and recipient shows that for PBSC it has no incidence at all on the result. This allows us to inscribe low weight donors for adults as well as for children. The blood volume treated and a regular flow during procedure seem to be bases of a successful PBSC collection. A poor CD34 cells mobilization doesn't allow a successful collection.
In conclusion, a majority of products don't correspond to the quantity requested. Ways of amelioration can and must be found.

**P831**

**The efficacy of the audovisual method used on the subject of stem cell transplantation for patient/donor informatory meetings**

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Background: The process of peripheral blood stem cell collection from patients and donors is similar to a normal blood donation, while the application is similar to blood transfusion. However, patients/donors carry anxiety concerning feeling of discomfort and pain during the process and possible side effects after the application. The use of audiovisual method during the informing process may reduce worries and may have a positive effect on the process of approval. To our knowledge, there has not been adequate study on this method of informing for stem cell transplantation (SCT). The effectiveness of the approval process was examined by performing the audiovisual informing method to the patients/donors.
Method: All the cases, that are implemented patient/donor informing process between the years 2008-2011, were included in the study. In the process, until the year 2010, an information Form including appropriate expressions to the laws and regulations were used. Since 2010, in addition to this form for patient/donor informing process, a DVD animation program that was produced for our center and offers several language options (Technical Media, Adana, Turkey) has been used. Informing process was evaluated by using Patient/donor Informing Functionality Form. Patient compliance was evaluated by the patient's physician, clinical directors and transplant nurse with compliance scoring.
Results: During the informatory process, within a total of 96 patient/donor, that the audiovisual method was not used, 9 (9.2%), within a total of 122 patient/donor, that the audiovisual method was used, 12 (9.4%), did not give consent to the procedure (P> 0.05). One patient (0,8%) despite the language problem, all the cases completed the informing process. While from the group, that gets the information without audiovisual method, 4 (4.2%) cases returned because of the some complaints related with hospital care, 2 (1.6%) within the used group, filled in complaint form. (P<0.05). The patient non-compliance score was found similar in the audio visual method used in compared to not-used group (0,505 *vs* 0,506; p>0.05).
Comment: The use of audiovisual method at the Patient/Donor Informatory Meetings for the purpose of stem cell collection and transplantation is an easy process to implement. It was found that using this method has not significantly influenced the approval process, however has been effective in reducing the patient/donor complaints.

**P832**

**The analysis of allogeneic transplants from unrelated donors, the impact of mismatches in the HLA system and identification of prognostic factors influencing these outcomes - the single-centre experience**

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We performed a retrospective analysis of allogeneic transplants from January 1995 - August 2010. A total of 614 patients, 223 were transplanted from related donors (203 from identical siblings (IS), 20 from mismatched relatives), 391 pts were transplanted from unrelated donors, of whom 200 were matched (MUD) 10/10, 112 with 9/10 single HLA antigen/allele mismatch and 79 with ≤8/10 mismatches. Different diagnoses were represented as follows: AML 182, ALL 70, MDS 103, CML 119, lymphoproliferation 95, myelofibrosis 39, SAA 5, FA1.
A more detailed analysis was then performed for unrelated transplants only, comparing MUD 10/10 *vs* 9/10 mismatch in the individual loci (A, B, C, DR, DQ) and *vs* ≤8/10 mismatched donors.
Results: The results of 10/10 IS and 10/10 MUD were comparable in all investigated parameters. When comparing the results of MUD 10/10 *vs* 9/10 (at any HLA locus), the groups did not differ in terms of OS, PFS and NRM. In transplants using donors ≤8/10, the OS, PFS and NRM were significantly worse than 10/10.
In terms of other prognostic factors, in the univariate analysis we identified: the presence of aGVHD significantly worsened OS and NRM, while cGVHD significantly improved OS and decreased relapse incidence without affecting the NRM. We observed less cGVHD when using ATG, less aGVHD when using RIC, less aGVHD when using bone marrow and significantly worse OS, PFS, NRM in advanced disease. Age (≥50 years and ≥60) had no impact in terms of OS, PFS and NRM. We observed significantly worse OS, PFS and NRM for the period 1995-1999. We did not demonstrate the influence of neither the AB0 mismatch nor sex mismatch.
In multivariate analysis, the following factors were identified as significant for OS: age, disease stage, diagnosis and HLA mismatch ≤8/10, for PFS: disease stage, diagnosis and HLA mismatch ≤8/10, for NRM: age, disease stage, HLA mismatch ≤8/10, for aGVHD: age and conditioning, for cGVHD: disease stage, conditioning and ATG administration.
Conclusions: Comparable results of transplants from IS and MUD. Probably acceptable mismatch 9/10 (small groups for meaningful comparisons). Worse results in mismatches 8/10 and more. Better results in patients with cGVHD. Acute GVHD does not bring any benefit. Administration of ATG reduces the incidence of cGVHD with no differences in OS. Much worse results of advanced disease in all monitored parameters.

**P833**

**Is the use of 9/10 HLA unrelated donors still acceptable in allogeneic haematopoietic stem cell transplantation for haematological malignancies? Comparison with transplants from 10/10 HLA unrelated donors and siblings**

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To evaluate the outcome of allo-HSCT from 9/10 HLA mismatched unrelated donors compared to those from 10/10 HLA identical unrelated donors and siblings; we retrospectively studied the outcome of 213 patients who received allo-HSCT for different hematological malignancies, 121 (57%) from HLA identical siblings, 63 (29%) from 10/10 HLA identical unrelated donors and 29 (14%) from 9/10 HLA mismatched unrelated donors between 2006 and 2011 at our institution. The different patient characteristics are described in Table 1. After HSCT,

engraftment was significantly lower in the 9/10 HLA group (90%) than in the 10/10 HLA group (95%) than in the sibling group (99%), (p=0.03); the cumulative incidence of acute GVHD≥2 at 3 months was 32% (23-41), 20% (15-26) and 27% (23-32) respectively; the cumulative incidence of extensive chronic GVHD at one year was 21% (13-30), 9% (5-13) and 17% (14-21) for the 3 groups respectively. After a median follow-up of 8 months (0-54) in the 9/10 HLA group, 10 months (0-60) in the 10/10 HLA group and 18 months in the siblings group, the median OS was 10 months (5-21), 18 months (11-NR) and 60 months (31-NR) respectively with a 2-years probability of 19% (8-44), 43% (31-59) and 63% (54-74) respectively. There was a higher but not significant relapse incidence at one year in the 9/10 HLA group compared to other groups while the TRM was significantly higher with a cumulative incidence at 1 year of 45% (35-55) *vs*. 33% (27-39) for 10/10 and 12%(9-15) for siblings, (p<0.001). In multivariate analysis, OS was negatively affected by unrelated donors [9/10 HR=5 (2.7-10), p=0.0001; 10/10 HR=2 (1.2-4), p=0.01], female donors [HR=2 (1.4-4), p=0.03] and disease status <CR1 or <chronic phase 1 [HR=3 (1.4-6), p=0.003]; while the TRM was negatively affected by unrelated donors [9/10 HR=9 (4-20), p<0.001; 10/10 HR=4 (1.2-10), p=0.03], female donors [HR=3 (1.2-7); p=0.01] and ABO minor incompatibility [HR=2.5 (1.2-5), p=0.01]. We showed that allo-HSCT from 9/10 HLA mismatched unrelated donors have a significantly worse OS than those from matched unrelated donors and siblings; this was mainly due to an increased TRM in this group. Patients in first CR or CP could benefit the more from matched or 9/10 unrelated allo-HSCT while the use of transplants from 9/10 HLA unrelated donors in patients not in CR1 should be limited to clinical trials.

**[P833]**

TABLE 1 : Patients characteristics and outcomes

| | HLA identical siblings group N=121 | 10/10 HLA unrelated group N=63 | 9/10 HLA unrelated group N=29 | p-value |
|---|---|---|---|---|
| **PATIENTS DESCRIPTION** | | | | |
| **Gender** | | | | |
| Male | 69 (62%) | 37 (59%) | 18 (62%) | NS |
| Female | 52 (38%) | 26 (41%) | 11 (38% | NS |
| **Age** | | | | |
| Median, years | 45 (18-66) | 44 (20-64) | 38 (19-61) | NS |
| **Disease** | | | | |
| Myeloid leukemia | 71 (59%) | 23 (37%) | 16 (55%) | 0,01 |
| AML / MDS | 54 / 7 | 15 / 0 | 13 / 2 | |
| CML / MPS | 3 / 7 | 2 / 6 | 1 / 0 | |
| Lymphoid leukemia | 50 (41%) | 40 (63%) | 13 (45%) | 0,01 |
| ALL / MM | 18 / 13 | 20 / 15 | 7 / 2 | |
| Lymphoma / CLL | 14 / 5 | 5 / 0 | 4 / 0 | |
| **Disease status** | | | | NS |
| CR1/CP1 | 40 (33%) | 23 (37%) | 9 (31%) | |
| CR2/CP2 | 26 (21%) | 7 (11%) | 7 (24%) | |
| <CR2/CP2 | 55 (45%) | 33 (52%) | 13 (45%) | |
| **Conditioning** | | | | NS |
| Full-intensity | 64 (53%) | 32 (51%) | 16 (55%) | |
| Reduced-intensity | 57 (47%) | 31 (49%) | 13 (45%) | |
| **Cell source** | | | | |
| PBSC | 55 (45%) | 28 (44%) | 13 (45%) | |
| BM | 66 (55%) | 35 (56%) | 16 (55%) | |
| **Sex-mismatching** | | | | 0,03 |
| Fd-Mr | 30 (25%) | 9 (14%) | 8 (28%) | |
| Md-Fr | 22 (18%) | 19 (30%) | 9 (31%) | |
| **CMV-mismatching** | | | | 0,07 |
| D- R+ | 25 (21%) | 13 (21%) | 8 (28%) | |
| D+ R- | 16 (13%) | 14 (22%) | 6 (21%) | |
| **ABO-mactching** | | | | |
| major | 22 (18%) | 21 (33%) | 6 (21%) | <0,0001 |
| minor | 7 (9%) | 20 (32%) | 8 (28%) | |
| **Interval diag-HSCT** | 16 months (2-219) | 14 months (4-136) | 9 months (4-87) | NS |
| **Median FU** | 18 months (0-60) | 10 months (0-48) | 8 months (0-54) | |
| **RESULTS** | | | | |
| **Egraftment** | 120 (99%) | 60 (95%) | 26 (90%) | 0,03 |
| **CI aGVHD at 3m.** | 27% (23-32) | 20% (15-26) | 32% (23-41) | NS |
| **CI cGVHD at 12m.** | | | | |
| Limited | 14% (11-18) | 11% (7-15) | 4% (0-8) | NS |
| Extensive | 17% (14-21) | 9% (5-13) | 21% (13-30) | NS |
| **Median OS** | 60 months (31-NR) | 18 months (11-NR) | 10 months (5-21) | <0,001 |
| **CI relapse at 12m.** | 17% (14-20) | 15% (9-20) | 26% (17-35) | NS |
| **CI TRM at 12m.** | 12% (9-15) | 33% (27-39) | 45% (35-55) | <0,0001 |

**P834**

**Double cord blood units allogeneic transplantation for haematological malignancies: study of the 3 partners matching variables on transplant outcomes**

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We retrospectively evaluated 31 patients, 19 males and 12 females with a median age of 36 years (range: 20-63) who received double CB-T for hematological malignancies at our institution between 2005 and 2011. There were 15 AML, 9 ALL, 2 CML, 1 Hodgkin disease, 1 NHL, 1 MM and 1 MDS;

at transplantation, 18 (58%) were in CR, 5 (16%) in PR and 8 (26%) in relapse, 16 patients received a myeloablative conditioning and 15 a reduced intensity one. When considering matching variables between the 2 CBUs and the recipient, for sex matching: in 10 cases, CBUs were both matched with the recipient, in 1 case both mismatched and in 20 cases, only 1 CBU was mismatched with recipient. For ABO compatibility, in 7 cases, CBUs were both compatible with the recipient, in 13 cases both were incompatible and in 1 case, only one was incompatible. For HLA matching, in 18 cases, both CBUs were 4/6 with the recipient and in 13 cases, only one CBU was 5/6. When considering HLA matching between the 2 CBUs, there were 7 with 3/6 HLA matching, 17 with 4/6, 6 with 5/6 and 1 with 6/6. After transplantation, 25 (81%) patients engrafted among them, 6 had mixed chimerism (presence of the 2 CBUs) and 19 had one dominant CBU. Non-engrafted patients were in relapse or progressive disease and received RIC before allo-HSCT. There were 13 patients who developed acute GVHD≥2 (8 grade III-IV) and 6 chronic GVHD (3 limited and 3 extensive). After a median follow-up of 6.5 months (range: 1-54), the median OS was not reached with a 1 year probability of 58% (95%CI: 42-80), only 3 patients relapsed. The cumulative incidence of TRM was 37% (95%CI: 28-46). A multivariate model that studied the different matching possibilities (sex, ABO and HLA) between the 2 CBUs together and then between the 2 CBUs with the recipient combined to CNT and CD34 cells number, showed that only the sex matching between the 3 partners can determine the dominant CBU later (p=0.04). In multivariate analysis, no factor impacted the engraftment while factors that impacted on OS were age [HR=1.1 (1.03-1.25), p=0.01], disease status at HSCT [relapse, HR=8.7 (1.4-52), p=0.01] and TNC number [HR=0.99 (0.98-1), p=0.02]. Age and TNC number also had a significant impact on TRM [HR=1.12 (1.02-1.23), p=0.01 and HR=0.99 (0.98-1), p=0.003]. In conclusion, sex matching between the 3 partners seems to play a role in the determination of the dominant CBU later and its installation in the recipient.

**P835**

**Allogeneic haematopoietic stem cell transplantation: full matched versus one antigen mismatched other than sibling relative donors**

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Objectives: To assess the effect of mismatched relative transplantation on Graft-versus-host disease (GvHD) and survival. Only 30% of patients who need transplantation have Human-Leukocyte Antigen (HLA) matched sibling donor. Therefore we are forced to use other related matched donors. The impact of HLA mismatching on transplantation outcome remains unclear.
Methods: This study included 95 patients (age: 5 months to 52 yrs) had undergone transplantation from 1992 to 2011 in our center. The patients were transplanted from other- related donors (not siblings), except haploidentical ones. Eighty percent of donors were fully HLA – matched and 20% were one antigen HLA –mismatched. Sixty three percent of donors were parents, 20% uncle/aunt, 12% grandparents, 4% cousins and 1% offspring. Patients were transplanted with bone marrow (58%) and peripheral blood (42%). The most common diseases were major beta-thalassemia, Fanconi anemia and acute leukemias.
Results: The median age of recipients was 6 years old. The male to female ratio was 58:37. All of the patients underwent successful graft procedures. The median time to neutrophil engraftment was 14 days (range: 9–62) in matched and 12 (range: 10-22) in mismatched group, (P=0.684). The median time to platelet engraftment was 21 (range: 9–75) and 18 (range:15-51) days (P=0.914) in matched and mismatched groups, respectively. The median onset of aGvHD was 10 (range: 3-38) in matched versus 8 days (range: 3-17) in mismatched group. The most common grade of aGVHD in the matched group was I, II while II, III in mismatched one. The cumulative incidence of acute GvHD, 17 days after transplantation was 43.4% (95% CI: 32%-54.2%) in matched and 78.9% (95% CI: 50.6%-92.1%) in mismatched (P<0.001), respectively. Median follow- up time was 317 days (range: 30-3632). The one- year overall survival was 77.7% (SE: 5.9%) in matched and 54.3% (SE: 12.5%) in mismatched group which was statistically significant (P=0.037). The most common causes of death were GvHD and infection in matched while GvHD and drug toxicity in mismatched group.
Conclusion: The overall survival was significantly less in mismatched group; however, they were first and second degree relatives of recipients. Although engraftment occurred a bit sooner (not significant) in mismatched group, the cumulative incidence of aGvHD was higher and onset time was earlier. The conditioning regimen and immunosuppressive therapy for mismatched transplantation must be revised.

**P836**

**Outcomes of transplantation with other-related- and sibling-donor stem cells in children with Beta-thalassaemia major**

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Objectives: To investigate the outcome of HLA-matched other-related HSCT and compare the results of HLA-matched sibling HSCT in Beta-thalassemia major pediatric patients.
Methods: We evaluated the outcome of transplantation in 25 consecutive children with severe thalassemia who underwent allo-HSCT from other-related-donor (group 1). We also compared the results obtained from 25 thalassemia major children who received sibling-donor cells for allo-HSCT (group 2). Group 2 was matched with group 1 with respect to age, gender of donor to recipient, thalassemia risk class, and the year of transplantation. Patients in both groups received the same busulfan-based conditioning regimen.
Results: The median age of patients was 9 years (72% male). In each group, 4 patients were classified into risk class 1; 13 risk class 2; and 8 risk class 3 of the Pesaro classification system. The most common source of stem cells in group 1 was bone marrow while in group 2 was peripheral blood stem cells (p=0.024). There was no significant difference between two groups for the time of neutrophil and platelet engraftments. The cumulative incidence of acute graft-versus-host disease by 21 days after transplantation for group 1 and group 2 was 60% (95% confidence interval (CI) of 38%-77%) and 40% (95% CI of 21%-59%), respectively (p=0.219). With a median follow-up period of 6 months (range: 1-43 months), the overall survival (OS) for thalassemia children in group 1 was 72.8% (SE 10.7%) compared with 85.3%% (SE 7.9%) for patients in group 2 (P=0.614). The difference between OS in two groups after adjustment for stem cell source was not significant (0.677).
Conclusion: The present study provides evidence supporting the consideration of other-related allo-HSCT as an appropriate therapeutic approach in thalassemia major children without matched-sibling-donors.

**P837**

**Occurrence of minor histocompatibility antigens' disparities and their impact on results of allogeneic haematopoietic stem cell transplantation from HLA-matched siblings**

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Introduction: The aim of this study was to investigate the occurrence and impact of immunogenic minor Histocompatibility Antigens' (mHA) disparities on outcome of allo-HCT from HLA-matched siblings.

Methods: Alleles of 11 mHA: HA-1, HA-2, HA-3, HA-8, HB-1, ACC-1, ACC-2, HwA-9, HwA-1, UGT2B17, HY were analyzed in search of immunogenic mismatches in 62 sibling donor-recipient pairs after allo-HCT performed between 2000-2008. The median age of pts was 38(14-59) yrs. Indication for allo-HCT was AML, ALL, MDS, CML and NHL 0.6(0.2-12.9) yrs from dgn. Preparative regimen was Bu+Cy or Treo+Flu. mHA alleles were determined using Dynal AllSet mHA typing kit and PCR-SSP method. Only immunogenic mHA mismatches established according to dbMinor Database with consideration of direction (HVG or GVH) and tissue distribution were evaluated.

Results: Relapse occurred in 15(24.2%) pts. Immunogenic mHA mismatches were identified in 42(68%) pairs, 20(32%) pairs showed no mHA immunogenicity. 24(39%) pairs had mHA mismatched in HVG direction [autosomal 10(16%), HY 12(19%), both 2(3%)] and 24(39%) in GVH direction [autosomal 12(19%), HY 9(15%), both 3(5%)]. Bi-directional mHA disparity was detected in 6(10%) pairs. Pts with GVH-directed HY disparity had lower both overall survival [OS 0.33(0.01-0.55) *vs* 0.68(0.52-0.79), p=0.011] and disease-free survival [DFS 0.33(0.01-0.55) *vs* 0.65(0.49-0.76), p=0.05] at 3 yrs than pts without this disparity. On contrary, the OS was higher in pts with autosomal mHA mismatched in GVH-direction [OS at 4 yrs: 0.76(0.35-0.91) *vs* 0.53(0.35-0.65), p=0.045]. Relapse incidence was increased in patients with HVG-directed disparity of either autosomal mHA [0.28(0.18-0.44) *vs* 0(0-0), p=0.032] or mHA with restricted distribution [0.29(0.18-0.45) *vs* 0(0-0), p=0.028].

Conclusion: Immunogenic mHA mismatches may be present after alloHCT from HLA-matched siblings and may impact OS, DFS and relapse rate. The influence of GVH-directed HY disparity helps to understand the role donor/recipient sex difference in transplantation. Continuation of the study is necessary to establish the associations of specific autosomal mHA mismatches with alloHCT results.

**P838**

**HLA-DPA1 mismatch is associated with decreased overall survival following unrelated donor haematopoietic stem cell transplantation**

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Aim: To investigate the impact of HLA-DPA1 and -DPB1 mismatch on clinical outcomes in a modern era of unrelated hematopoietic stem cell transplantation (HSCT).

Methods: Retrospectively, 380 consecutive patients (median age 39, range 0.5-67 years) who underwent HSCT using HLA -A, -B, and -DRB1 allele level-matched unrelated donors at our centre during 1995-2010, were included in the study. Most patients underwent HSCT in the treatment for hematological malignancies (81%), whereas 7% and 12% of all patients were transplanted in the treatment for non-hematological malignancies and non-malignant disorders, respectively. HLA typing using PCR-SSP (Olerup-SSP) or PCR-SSO on a Luminex platform (One Lambda) was performed and revealed 55 HLA-DP (A1 and B1) matched and 325 mismatched donor pairs. HLA-DPA1 and HLA-DPB1 mismatch was noted in 35% and 85% of all recipient donor pairs, respectively.

Results: HLA-DP (A1 or B1) mismatch compared to DP match did not affect the incidences of acute graft-versus-host disease (GVHD) grades II-IV (33% *vs*. 27%; P=0.20), or grades III-IV (6% *vs*. 8%; P=0.87). Furthermore, the incidence of chronic GVHD was similar (28% *vs.* 27%; P=0.49), and there was no impact on patient overall survival using DP mismatched compared to DP matched donors (55% *vs*. 50%, P=0.72). However, in separate analysis DPA1 mismatch was associated with decreased overall survival (HR 1.50, P=0.02), when compared to matched recipients irrespective of HLA-DPB1 and HLA-C match. In univariate analysis, HLA-DPA1 mismatch was associated with increased risk of transplant-related mortality (P=0.04), although incidences of acute and chronic GVHD were similar. Furthermore, a tendency to an increased risk of infection-related mortality was noted in patients mismatched for HLA-DPA1 (P=0.07).

Conclusions: This study suggests that HLA-DPA1 mismatch is associated with inferior survival, which may be caused by an increased risk of infection-related mortality. At present there are few publications available, which investigate the role of DPA1 and DPB1 mismatch separately, and future studies to uncover the importance of DPA1 and DPB1 compatibility in unrelated HSCT are warranted.

**P839**

**Mismatched haematopoietic progenitor cell transplantation is an effective therapy for patients with high-risk haematopoietic neoplasms**

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Objective: To study the efficacy and safety of human leukocyte antigen (HLA) mismatched hematopoietic stem cell transplantation (HSCT) on high risk hematopoietic neoplasms.

Methods: We analyzed 39 consecutive mismatched allogeneic hematopoietic cell transplantation performed from 2005 to 2011. Median age was 38 years (range, 12-63 years). Patients had been diagnosed with 10 acute myeloid leukemia, 9 acute lymphoblastic leukemia, 2 biphenotype leukemia), 4 myelodysplastic syndromes, 4 myeloproliferative neoplasms, 3 chronic lymphocytic leukemia, 4 lymphoma, 2 aplastic anemia.

The median number of prior regimen was 2 (range, 0-5). 8 patients (21%) had received a previous hematopoietic cell transplantation (7 autologous and 1 allogeneic) and 13 patients had an advanced/active disease.

The Sorror Comorbidity index was intermediate/high in 17 patients (44%). A total of 31 patients were conditioned with high-dose myeloablative therapy and 8 patients were conditioned with reduced-intensity regimens. Anti-T-cell globulin (ATG) was part of the conditioning therapy in 13 patients.

Standard GVHD prophylaxis consisted of calcineurin inhibitor and a short course of MTX in 28 patients or calcineurina inhibitor with mycophenolate in 11 patients. 30 (76%) patients were transplanted with bone marrow and 9 (23%) patients were transplanted with peripheral blood stem cells. All patients were HLA typed by molecular methods for 10 alleles (HLA-A, -B and -C as well as -DRB1 and -DQB1). HLA antigens were 1-loci-mismatched in 30 (77%) patients and 2-loci-mismatched in 9 (23%).

Results: 37 patients achieved primary engraftment. 2 patients experienced a primary graft failure. By 30 days post-HSCT, complete donor chimerism was observed in 33 patients and mixed chimerism in 2 patients. The incidences of acute graft versus host disease (aGVHD) grade II-IV and aGVHD grado III-IV were 41% (n=16) and 18% (n=7), respectively. 7 of the patients with acute GVHD grade III-IV showed steroid resistant. The incidence of chronic GVHD in 31 evaluable patients was

26% (n=8). Five patients developed extensive chronic GVHD. After a median follow-up of 22 months (range, 3-77 months), 21 (55%) patients are alive. 15 patients of the 39 patients transplanted have died, mostly related to aGVHD (n=8) and relapse disease (n=5).
Conclusion: Mismatched HSCT is a feasible and safe option for patients without sibling identical donors.

**P840**
**HLA-matched sibling donors share the same genotypes of killer immunoglobulin-like receptors with patients more frequently than not HLA-identical siblings**
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Natural killer (NK) cells are the first lymphocyte subset, which reconstitute the peripheral blood after allogeneic hematopoietic stem cell transplantation. NK cells are implicated in bone marrow engraftment, the mediation of graft-versus-leukemia effect and in the suppression of graft-versus-host disease. Killer immunoglobulin-like receptors (KIR) of NK cells recognize the absence of self MHC class I molecules and define immune "self". The KIR genes are located on chromosome 19q13.4, and the genes of their HLA ligands are located on chromosome 6, so these genes segregate independently of each other. Despite the independent segregation of genes encoding KIR and HLA, there are evidences of some kind of co-evolution. If there is no dependence one could expect that KIR genotype identity between patient and sibling (potential donor of hematopoietic stem cells) in HLA-matched pairs will be the same as in not HLA-identical pa-tient/sibling pairs. The objectives of our study were to investigate the frequency of KIR genotype identity in HLA-matched patient/sibling pairs and not HLA-identical pairs.
Methods: 68 patients with different hematological diseases and their 68 siblings were studied. They were divided into two groups: first group consisted of 33 HLA-identical patient/sibling pairs and the second group consisted of 35 not HLA-identical patient/sibling pairs (22 HLA-haploidentical pairs and 13 pairs different for the both HLA-haplotypes). KIR genotyping was done using KIR Genotyping SSP Kit (Invitrogen, WI, USA). Differences in the frequencies of pairs carrying the same and the different KIR genotypes for patient and sibling in each group were estimated by Fisher exact test.
Results: In the first group there were 48.5% of KIR-genotype identical of pairs, in the second group there were only 37% of KIR-genotype identical pairs ($p<0.05$). 51.5% of the pairs in the first group *vs*. 63% of pairs in the second group had different KIR genotypes. The frequencies of KIR-genotype identity in HLA-haploidentical pairs and in pairs different for the both HLA-haplotypes were the same (36.5% and 38.5%, correspondingly).
Conclusion: HLA matched sibling donors share the same KIR genotypes with patients more frequently than not HLA-identical siblings. Nearly the half of HLA matched sibling donors have the same KIR genotypes as the recipients. The data do not allow excluding of the possibility of HLA influence on the KIR gene segregation.

**P841**
**A new score to determine the probability of finding an HLA-identical unrelated donor: a promising efficient time and cost-saving method**
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To refine and accelerate the process of donor search by combining the results of Tiercy and EasyMatch programs and define a new score for donor finding probability, in order to be time- and cost-efficient, we retrospectively analyzed 104 adult and 34 pediatric patients transplanted between 2009 and 2011 after finding an unrelated donor (identical or not) or cord blood units. Firstly, we analyzed the HLA characteristics of each patient as previously described by Tiercy *et al.* to provide a HLA score with Low, intermediate or high probability to identify a suitable identical donor. Then, we used the EasyMatch software which realize a "qualitative" analysis that consist on checking that each HLA recipient phenotype was found among all possible pair wise combinations of 2 haplotypes of the different sets of haplotypes. Various "quantitative" analyses calculated the likelihood associated to each recipient phenotype for a given set of HLA genes, in a given population, at low versus high resolution typing. The EasyMatch software gives for each patient a number of potential donors sharing the same phenotype as the patient.
Results: Our 138 patients were classified in 5 different categories (A to E) according to the combined results of the HLA score (Tiercy) and the EasyMatch software (Table).

**[P841]**

| | Tiercy Score | EasyMatch Number of potential donors | Patients | % of patients who found a donor and received allo-HSCT | |
|---|---|---|---|---|---|
| A | Low | 0 or < 1 | 59 | No HLA identical donor or cord blood unit : 100% | p=0,0003 |
| B | Low | > 1 | 5 | Non HLA identical donor or cord blood unit: 60% | p=ns |
| C | Intermediate | < or = 5 | 23 | No HLA identical donor or cord blood unit: 56% | p=0,0008 |
| D | Intermediate | > 5 | 16 | HLA identical donor: 87% | p=ns |
| E | High | > 1 | 35 | HLA identical donor: 100% | |

The results of the combination of the two methods allowed the definition of a new scoring system applicable to each patient and an economic strategy for an active search of donor. Score 0 (group A): 0% of chances to identify a 10/10 identical donor for the recipient. The choice of the source will be defined considering the HLA characteristics of the recipient; in case of class I rare allele or rare HLA-BC linkage disequilibrium; a cord blood unit will be easier and more rapidly available. A complementary help should be given by an associated analysis with 4-digit haplotypes as defined by Maiers (Human Immunology 2007, 68; 779-788). Score 1 (groups B and C): 50% of chances to identify a suitable 10/10 identical donor. Score 2 (groups D and E): from 75 to 100% of chances to identify a suitable 10/10 identical donor.
In conclusion, the use of this new scoring system allows time and cost spare. In case of low chance to find a donor, physicians can have a fast redirection to find another treatment alternative in order to keep an optimal results.

**P842**

**Anti-HLA antibodies after allogeneic haematopoietic stem cell transplantation from HLA-mismatched unrelated donors**

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Objectives: Although anti-human leukocyte antigen antibodies (anti-HLA Abs) are important factors responsible for graft rejection in solid organ transplantation, their role in allogeneic hematopoietic stem cell transplantation (allo-HSCT) is unknown. The purpose of this study was to define the incidence and profiles of anti-HLA Abs produced after allo-HSCT from HLA-mismatched unrelated donors and their impact on allo-HSCT results. Methods Anti-HLA A, B, C, DR, DQ, DP Abs were identified in sera collected from 30 recipients of allo-HSCT from HLA-mismatched unrelated donors. Indication for allo-HSCT was CML (17), AML (7), ALL (4), SAA (2pts). Preparative regimen was myeloablative: treosulfan-based in 16, busulfan/cyclophosphamide(Cy) in 8, TBI/Cy in 5 and Cy/ATG in 1 pt. Standard GVHD prophylaxis consisted of cyclosporine, methotrexate and pre-transplant ATG (13pts) or thymoglobulin (17pts). Source of cells was bone marrow (17pts) or peripheral blood (13pts). Sera were collected 115 to 2156 days after allo-HSCT. Two methods were used for detection of anti-HLA Abs: 1- LabScreen microbeads coated with purified Class I and II HLA antigens; 2- automated DynaChip assay which uses microchips bearing HLA class I or II antigens.
Results: Anti-HLA Abs were detected in 17(57%) and were not in 13(43%) pts. Anti-HLA Abs class I, II or both were detected in 5(29%), 5(29%) and 7(42%) pts, respectively. Abs were directed against HLA- A,B,C,DR,DQ. Neither anti HLA-DP nor donor-specific anti-HLA Abs were detected, although recipient-specific anti-HLA Abs were detected in one pt. Acute GVHD was observed in 14(82%) or 6(46%) pts with or without anti-HLA Abs: grade I,II,III and IV respectively in 8,4,1,1 or 4,1,0,1 pt. Chronic GVHD occurred in 10(59%) or 5(38%) pts: limited in 8 or 3 and extensive in 2 or 2 pts. Acute infections (sepsis, pneumonia) have developed in 5(29%) or 5 (38%) pts; relapse occurred in 3(17%) or 5(38%) pts with or without anti-HLA Abs. 100% donor chimerism was achieved by 16(94%) or 13(100%) pts and decreased to 25% in 8(50%) pts with Abs and to 58% in 4(30%) pts without anti-HLA Abs.
Conclusions: These preliminary results indicate that anti-HLA Abs can appear post-transplant in mismatched allo-HSCT recipients and may be potentially responsible for the occurrence of post-transplant complications.

**P843**

**The probability of finding a matched related donor for haematopoietic stem cell transplantation in immediate and extended families in Jordan**

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Purpose: To evaluate the probability of finding HLA-matched related donor for hematopoietic stem cell transplantation (HSCT) in Jordan which represent an example of developing country in general and Arabic country in particular, as data in this field are scanty.
Patients and Methods: Retrospective review of medical records of patients and their families who did HLA typing study at King Hussein Cancer Center between January 2003 and November 2011 was performed.
Results: A total of 1200 patients were included. 65% (n=810) were pediatric (<18 years), and 35% (n=440) were adults. Fifty six percent (n=672) had malignant diseases, including acute leukemia 69% (n=470), chronic leukemia 9.4% (n=63), lymphoma 12.4% (n=83), myelodysplastic syndrome 3% (n=20), myeloproliferative diseases 3% (n=19), plasma cell disorders 1.1% (n=8), and solid tumors 0.8% (n=5). While 44% (n=528) hand non-malignant disorders including haemoglobinopathy 67.5% (n=353), bone marrow failure diseases 17.4% (n=92), primary immunodeficiency disorders 10.7% (n=56), and inherited metabolic diseases 4.4% (n=23).
The Probability of finding matched related donor from immediate and extended families was 65.4% (n=817), with 11.1% (n=91) having non-siblings related donors from extended family search.
The chance of having a donor was 61% (n=491) for pediatric and 74% (n=322) for adult patients. It was 71% (n=477) for patients with malignant diseases, and 61% (n=321) for patients with non-malignant disorders. Median family size was 5(range of 1 to 14); it was 5(1-14) for patients with donors and 4(1-10) for patients without donors. The average number of donors was 2(range of 1-6) for all patients with donor.
Conclusion: The chance of finding matched related family donor for HSCT in Jordan is much higher than Western countries and Asia (65% versus 25%). We expect a similar trend in other developing and Arab countries. This high probability was observed in both malignant and non-malignant (inherited) disorders; which might highlight important genetic factors in our patients with malignant disorders.
Our data provided directional considerations of searching for matched donor,and may have impact on HSCT outcome. We recommend screening extended family donor prior to unrelated donor search. Also, ethnic pool in Jordan is likely homogenous, which is reflected by favorable chance of finding a matched donor. This provides strong incentive to establish national and regional unrelated donor registries and cord blood banks.

**P844**

**Unmanipulated related haploidentical transplantation can achieve comparable outcomes to identical sibling transplantation for paediatric patients with high-risk acute leukaemia**

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Objective: To answer whether the outcomes of related haploidentical HSCT was inferior to those of identical sibling HSCT in pediatric patients 18 years or less with high risk acute leukemia that were indicated for allogeneic hematopoieyic stem cell transplantation (HSCT).
Methods: 286 patients with high risk acute leukemia between June of 2000 and December of 2010 were enrolled. The order

of preference of donor selection was identical sibling donor (ISD), unrelated donor or umbilical cord blood, followed by related haploidentical donor (HID). Conditioning for ISD group was modified BuCy2. ATG was used for patients with HID. Cyclosporine A, mycophenolate mofetil and short-term methotrexate were used for graft-versus-host disease (GVHD) prophylaxis. Donors were primed with granulocyte-colony-stimulating factor (G-CSF). G-CSF mobilized bone marrow (G-BM) and peripheral blood cells (G-PB) were used as graft. All patients in HID group received G-CSF from day 6 to myeloid recovery after transplant.

All pediatric patients with refractory, relapsed acute leukemia and beyond CR2 were considered to receive allo-HSCT. High risk acute leukemia in CR1 or CR2 was defined as: poor and intermediate risk ALL, all AML except for those with good risk chromosomes.

Surviving patients were followed up to 31 October, 2011. Study endpoints were leukemia-free survival (LFS), engraftment, GVHD, relapse, overall survival (OS) and transplantation related mortality (TRM).

Results: Patients with less than 90% in Play-Performance Scale before HSCT had a higher risk for chronic GVHD (relative risk [RR]=4.29, P=0.00) and relapse (RR=3.83, P=0.00). Patients in CR1 or CR2 without t(9;22) or t(4;11) had lower risk for relapse (RR=0.41, P=0.01). Patients with acute GVHD 2-4 and more than 90% score in Play-Performance Scale before transplant had superior LFS (RR=2.58, P=0.01; RR=3.428, P=0.00) and OS (RR=3.37, P=0.00; RR = 3.95, P=0.00). Thirty-three patients died of transplantation related toxicities. Fifteen (45.5%) died of infections, nine (27.3%) patients died of GVHD. Four (12.1%) patients died of post transplantation lymphoproliferative disorder (PTLD).

Conclusion: Comparable outcomes of unmanipulated haploidentical HSCT were showed for pediatric patients with high risk acute leukemia with those of identical sibling HSCT in terms of LFS, as well as engraftment, acute GVHD grade 3-4, relapse, TRM and overall survival.

**P845**

## Haploidentical T-replete PBSC transplantation with reduced-intensity aplastic phase conditioning and post-transplantation cyclophosphamide for refractory myeloid leukaemias: a pilot study

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Allograft is often futile in refractory AML patients with high bone-marrow blast count. We conducted a pilot study incorporating cytoreduction with high dose cytosine and mitozantrone from day -14 to -12 along with alemtuzumab 10 mg x 3, followed by a reduced intensity conditioning and haploidentical graft, which was mobilized PBSC rather than bone marrow. The donors selected were either mother or NIMA mismatched siblings irrespective of NK cell alloreactivity. The conditioning protocol was developed based on Johns Hopkins regimen of Fludarabine and low-dose Cyclophosphamide pre-transplant with escalating dose Melphalan 70-140 mg/m$^2$ replacing 2 Gy TBI. Post-transplant Cyclophosphamide was administered 72hrs after infusion of the graft at 50 mg/kg twice at 24 hrs interval. Cyclosporine and MMF were started 24 hours after the last dose of Cyclophosphamide.

5 patients (median age-16, 5-32) underwent Haplo-HCT with a median BM blast count of 50% (20-80%) having failed at least two lines of treatment. The conditioning was tolerated without any major non-hematological toxicity, however all patients were severely neutropenic and febrile before day 0. The median CD34 was 7.06 x 10$^6$/kg (range 5.05-11.06) and CD3 was 36x 10$^7$/kg (range 8-79).

All patients engrafted with neutrophils >500/µl on day +14 (range 12-17) and platelet count >20,000/µl on day +15 (range 9-38) with >95% donor chimerism on day +30 with morphological CR. aGVHD grade II developed in one and the first two patients developed grade III aGVHD following DLI on day 90-100 due to increasing blast count in bone marrow with falling

**[P844]** **Table 1. Patient-, disease-, and transplantation-related characteristics**

| Characteristics | Total n (%) | HID group n (%) | ISD group n (%) | *P* |
|---|---|---|---|---|
| **No. of patients** | 229 | 188 (82.1) | 41(17.9%) | |
| **Median age (range), yr** | 15 (3-18) | 15 (3-18) | 14 (5-18) | |
| Age at transplantation, yr | | | | |
| Younger than 12 | 68 (29.7) | 56 (29.8) | 12 (29.3) | |
| 12-18 | 161 (70.3) | 132 (70.2) | 29 (70.7) | |
| **Male sex** | 153 (66.8) | 119 (52.0) | 34 (82.9) | 0.017 |
| **Play-Performance Scale before transplantation,** <90% | 70 (30.6) | 63 (33.5) | 7 (17.1) | 0.041 |
| **Diagnosis before transplantation** | | | | |
| Acute mylogenous leukemia | 82 (35.8) | 69 (36.7) | 13 (31.7) | |
| Aacute lymphoblastic leukemia | 147 (64.2) | 119 (63.3) | 28 (68.3) | |
| **Indications for transplantation** | | | | |
| Refractory/Relapsed | 27 (11.8) | 23 (19.5) | 4 (9.8%) | 0.000 |
| Beyond the second remission | 10 (4.4) | 10 (5.3) | 0 | |
| Second remission | 51 (22.3) | 51 (27.1) | 0 | |
| Poor risk | 30 (13.1) | 30 (16.0) | 0 | 0.000 |
| Intermediate Risk | 21 (9.2) | 21 (11.2) | 0 | 0.000 |
| Initial remission | 141 (61.6) | 102 (86.4) | 39 (95.1) | |
| Poor risk | 89 (38.9) | 67 (35.6) | 22 (53.7) | |
| Intermediate risk | 52 (22.7) | 35 (18.6) | 17 (41.5) | |
| **Median mononuclear cells (×10$^8$/kg)** | 7.87 | 7.92 | 6.80 | |
| Median CD3+ cell (×10$^8$/kg) | 1.73 | 1.88 | 1.24 | |
| Median CD34+ cells (×10$^6$/kg) | 2.44 | 2.46 | 2.23 | |
| CD34+ cells < 2.45×10$^6$/kg, n. (%) | 197 (86.0) | 163 (86.7) | 34 (82.9) | |

[P844]

**Table 2. Clinical outcomes of related haploidentical HSCT compared with identical sibling HSCT for pediatric patients with acute leukemia**

| Group | Haploidentical n=188 | Identical sibling n=41 | *P* value |
|---|---|---|---|
| **Neutrophil recovery** | | | |
| Case (%) | 187 (99.5%) | 41 (100%) | |
| Time: median (range) | 13 (9-28) d | 16 (9-24) d | |
| **Platelets recovery** | | | |
| Case (%) | 179 (95.2%) | 40 (97.6%) | |
| Time: median(range) | 16 (70-180) d | 14 (7-26) d | |
| Acute GVHD* | | | |
| Grade 2 - 4 | 38.8% (± 3.7%) | 10.4% (± 4.2%) | 0.01 |
| Grade 3 - 4 | 12.5% (± 2.6%) | 7.9% (± 3.6%) | 0.34 |
| Chronic GVHD | 21.1% (± 3.2%) | 18.2% (± 6.2%) | 0.71 |
| Extensive | | | |
| chronic GVHD | 15.0% (± 2.8%) | 6.0% (± 4.1%) | 0.14 |
| Relapse* | 24.5% (± 3.5%) | 25.5% (± 7.6%) | 0.83 |
| LFS* | 59.5% (± 4.0%) | 59.0% (± 8.4%) | 0.78 |
| OS* | 60.0% (± 4.4%) | 59.0% (± 8.7%) | 0.84 |
| TRM* | 10.0% (± 4.8%) | 16.1% (± 2.6%) | 0.36 |

GVHD*: graft-versus-host disease, cumulative incidence with competing risk; Relapse*: cumulative incidence with competing risk; LFS*: leukemia-free-survival, probability; OS*: overall survival, probability; TRM*: transplantation related mortality, cumulative incidence with competing risk.

donor chimerism. These patients had received the lowest dose of melphalan (60 and 70 mg/m$^2$) in the study. Both achieved CR with full donor chimerism post-DLI. Subsequent patients received melphalan at 100 and120 mg/m$^2$ and remained in CR at a median follow-up of 120 days (100-180 days). TRM was due to MDR GNB sepsis in one patient who developed post-transplant HLH and in another whilst on treatment for post-DLI GVHD.
In conclusion, this approach was successful in achieving donor engraftment and achievement of CR in all 5 patients with high marrow blasts. This was probably attributed by the GVL effect generated by haploidentical PBSC and effective cytoreduction immediate pre-transplant. Haploidentical PBSC in combination with post-transplant cyclophosphamide is not associated with increased acute GVHD and might be more effective for such advanced leukemias.

# Cellular, Gene Therapies and Cytokines

**P846**

**Clinical use of fast DCs transfected with hTERT and Survivin mRNA – an effective and simplified cancer vaccine approach**

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Since 2000, 170 patients have been treated with an in-house developed cancer vaccine protocol. Initially dendritic cells (DCs) were transfected with autologous tumour mRNA. Currently we combine mRNA from the tumor (when available), hTERT and Survivin. Overall 50% of the patients have mounted specific immunresponses following vaccination and this is related to improved clinical outcome. Until recently DCs were prepared over 7 days. Previously we have shown in small scale experiments that production of DCs can be done in 3 days (fast DC) and that these vaccines appeared to be as efficient as those produced over 7 days when tested *in vitro*. Recently we have translated our small-scale fast DCs results into the clinic and here we report our experiences by this new protocol.

Monocytes were isolated by elutriation of the leukapheresis products from 16 patients and the enriched monocytes were frozen. Following thawing and washing the cells were transferred into gas permeable Teflon bags and cultured with CellGro DC medium supplemented with GM-CSF and IL-4 at a cell concentration of $1x10^6$ cells/ml. After 48 hours maturation cocktail (GM-CSF, IL-4, TNF-alpha IL-1 beta and PgE2) was added and the cells were incubated for additional maximum 24 hours. Mature Fast DCs were then transfected with hTERT mRNA and Survivin mRNA by electroporation. After over night culture in Teflon bags with CellGro DC medium, without any cytokines added, the fast DC vaccines were frozen and stored until use. About 50% of the total cells at start of culture were recovered at the end of the production. Fast DCs had strong expression of HLA-DR, CD83, CD86 and CCR7 and weak expression of CD1a. Viability was in all cases <90%.
The fast DC vaccines have been given to 13 metastatic patients with recurrences after standard therapy (pancreatic cancer, prostate cancer, ovarian cancer). So fare 10 patients have been evaluated and among them eight show antigen specific immunresponses against hTERT and Survivin. Immunresponses was directly related to improved clinical outcome and as will be shown several of the patients have durable remissions. We conclude that our new fast DC protocol not only is facilitating the production of DCs but also appear to give higher specific immunresponses that the standard 7 days produced DCs.

**P847**

**T-helper-1-driven polyclonal T-cell populations for adoptive immunotherapy against tumour associated antigens**

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Adoptive T cell therapy has been shown a promising option to treat patients with malignancies. In contrast to vaccinations, T cells for adoptive T-cell therapy are generated *ex vivo* to be re-infused into the recipient. This enables treatment of immunocompromized hosts and use of allogeneic T cells to exploit graft versus tumor effects. Adoptive T-cell therapy involving CD4+ T-helper cells (Th cells), intends to induce sustained T-cell responses *in vivo*. The Th1 cytokine interferon-gamma (IFN-gamma) has not only an effect in orchestrating cytotoxic T-cell responses, IFN-gamma by itself has antitumor effects. Transferring T cells into a lymphopenic host furthermore offers access to homeostatic cytokines and elimination of regulatory T cells (Tregs). The aim of our study was the translation of a preclinical protocols into a GMP conform clinical scale protocol to generate specific T cells for adoptive T-cell therapy against tumor associated antigens (TAA). Large scale generations of NY-ESO-1 or Survivin specific T cells was performed according to current GMP regulations in a GMP facility. In brief, peripheral blood mononuclear cells from healthy donors were primed with an overlapping TAA 15-mer peptide mix. The priming was done in the presence of IL-7 and IL-2. T cells were enriched using IFN-gamma capture technique and expanded for two weeks in autologous culture conditions with IL-7, IL-15 and IL-2. The T-cell products showed high specificity, cytotoxic capacities of T cells and the Th1 cytokines IFN-gamma; and TNF-alpha. Tolerogenic cytokines like IL-10 were absent and Tregs excluded. Both, CD4+ and CD8+ T cells with an effector memory phenotype proliferated in response to TAA. The T-cell product did not include alloreactive T cells. In summary GMP-conform generation of TAA specific T cells was established. Although tumor associated antigens are potential self antigens, it is possible to induce a functional Th1 response in peripheral blood T cells from healthy donors. Adoptive T-cell therapy against tumor associated antigens could have implications for multiple tumor entities in autologous as well as allogeneic treatment approaches.

**P848**

**Low numbers of regulatory T-cells (% CD4 T-cells) within the haematopoietic stem scll graft are associated with poor engraftment and worse overall survival following allogeneic haematopoietic stem cell transplantation**

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The composition of the haematopoietic stem cell (HSC) graft may be critical to immune reconstitution and successful allogeneic haematopoietic stem cell transplantation (HSCT). In this prospective study, the composition of 101 HSC grafts was analysed and in particular, investigated whether CD3+CD4+CD25+ FOXP3+CD127dim regulatory T-cells (Treg) (relative (%CD4) and absolute counts) were significantly associated with engraftment, CMV reactivation, relapse, non-relapse mortality (NRM) and overall survival (OS). The median CD34, Treg, CD3, CD4 T-cell and NK cell count transplanted was $6.1x10^6$/kg (0-19.8), $4.6x10^6$/kg (0.2-20.6), $274x10^6$/kg (21-801), $170x10^6$/kg (12-694) and $31x10^6$/kg (2-101) respectively. Tregs represented 2.94% (0.81-8.56%) of the CD4 T-cells. Absolute Treg counts showed a strong correlation with T-cells but only a weak correlation with CD34 counts (Spearman coefficient 0.69 (P<0.0001) and 0.23 (P=0.02) respectively). In univariate analysis, patients transplanted with HSC grafts containing Tregs (%CD4) in the lowest quartile (<2.17% CD4 T cells) had inferior neutrophil and platelet engraftment (76.0 v 96.0% at day 40; P=0.03 and 68.0 v 85.5%; P=0.03 respectively), higher non-relapse mortality (38.6 v 16.0% at 2 years; P=0.02) and lower overall survival (41.1 v 67.8% at 2 years; P=0.007). However, Tregs were not significantly associated with CMV reactivation or disease relapse. In multivariate analysis, low Tregs (%CD4) (HR=0.57 (0.35-0.94); P=0.02) and low CD34 counts (HR=0.39 (0.25-0.59); P=8.4x 10-6) were independent predictors of neutrophil engraftment. Similarly, low Tregs (%CD4) (HR=0.53 (0.32-0.89); P=0.02) and low CD34 counts (HR=0.32 (0.20-0.52); P=2.3x10-6) were independent predictors of platelet engraftment. Low Tregs (%CD4) were also independently associated with non-relapse mortality and reduced overall survival (HR=3.04 (1.15-8.04); P=0.03 and HR=2.58 (1.24-5.35); P=0.01 respectively). Other variables independently associated with NRM and reduced OS were low NK counts (HR=4.19 (1.55-11.30); P=0.004 and HR=3.08 (1.47-6.46); P=0.003 respectively) and recipient positive CMV status (HR=2.94 (1.12-7.70); P=0.03 and HR=2.54 (1.21-5.31); P=0.01.) This data supports the hypothesis that graft composition, and in particular Tregs (%CD4), may influence transplant outcomes (engraftment, NRM and OS) and suggests that Treg cell therapies may be a useful strategy to improve allogeneic HSCT.

**P849**

**Fully automated washing of cryopreserved PBSC in a multicentre study**

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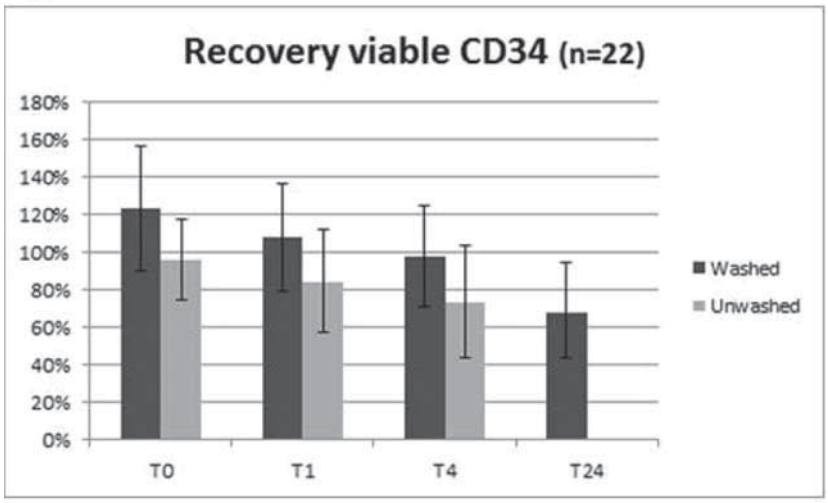
Cryopreservation of Hematopoietic Stem Cells is a standard procedure for autologous transplantation and allogeneic cord blood transplantation. The graft is usually infused through a central venous line following bedside thawing and without further manipulation, resulting in some toxicity related to a number of product components. Washing a thawed Peripheral Blood Stem Cells (PBSC) graft before infusion in a cell therapy facility involves the risk of losing progenitors and additional

[P849] Figure 1:




costs, mainly because procedures are carried out manually and are time-consuming and operator-dependent. A prospective, observational trial was designed in 4 European Centers (Basel, Florence, Marseille and Murcia) to assess the reproducibility and feasibility of a fully automated, closed system for washing thawed cellular products. We report here the outcome of the pre-clinical phase.
Methods: Cryopreserved PBSC Units from patients who permanently lost the indication to the transplant were used for this pre-clinical study. An ISHAGE-modified flow cytometry method was developed to improve the assessment of CD34+ cells after thawing. Units were thawed at 37°C and split in two fractions: one was washed with the Sepax® 2 system (Biosafe, Eysins, CH), the other left in the cryopreservation medium. Clinical grade hydroxyethyl starch (Voluven® 6%, Fresenius, Germany) was validated and used as washing solution. A sample was drawn in parallel from both washed and unwashed fractions at the end of the process and thereafter at 1, 4 and 24 hours, respectively; both fractions were kept at room temperature. Cell characterization and clonogenic tests were carried out at each time-point. Data and flow-cytometer scatters were centralized for the analysis.
Results: The washing procedures were carried out without any problem in all Centers. Recovery of viable CD34+ cells (as compared to post-thaw data) is shown in Fig 1. At all time-points, recovery of washed CD34+ cells was higher than in the unwashed controls. A recovery above 100% after washing may be related to the downregulation of CD34 antigen by DMSO. Recovery of viable CD34+ cells was above 50% at 24hrs after the procedure, confirming the stability of the washed product.
Conclusion: Sepax® 2 is an efficient and safe automated device to wash thawed PBSC graft. Its feasibility and reproducibility was confirmed in an International, multicenter, research protocol. The washed product is stable and clinically usable for several hours after thawing.

**P850**

**Expansion of CD3 negative CD56 positive NK cells for repeated clinical application in a gmp compliant process using a novel bioreactor system**

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Background: Natural Killer (NK) cells show high cytotoxic activity against tumour cells, act as major effector population of antibody dependent cytotoxicity (ADCC), and contribute to the anti-leukemic effect in haploidentical transplantation by killing target cells, which lack MHC class I molecules, without inducing graft-versus-host-disease. The haploidentical transplantation program established at our institution1 comprises CD34-selected stem cells and an additional transfer of high numbers of CD3-/CD56+ NK cells. With a novel bioreactor system we succeeded in mass expansion of effective NK cells under GMP compliant conditions, allowing high dose and repeated treatments.
Methods: NK cells were obtained from peripheral blood mononuclear cells from healthy donors (n=6) by CD56+selection and CD3-depletion by magnetically labelled bead technology. Suspensions of purified CD56+CD3-NK cells (Median 2x10e6 cells; CD3+ T cells <3%) were transferred into the disposable bioreactor system. After sedimentation, NK cells were cultured under continuous laminar flow for a median of 28 days (range 25–40) without feeder cells. Feeding rates, pH and pO2 values as well as temperature were controlled automatically by the bioreactor system. Harvested NK cells were analysed for puritiy, NK cell phenotype and natural cytotoxicity against K562 cell line (in standard calcein cytotoxicity assay). ADCC was analysed using the anti-CD20-antibody Rituximab and the CD20+ lymphoma cell line Raji.
Results: CD3-/CD56+ cells expanded 250 to 400-fold. Further expansion by more than 100-fold was possible in a larger disposable bioreactor. The mean NK cell purity was >95% (range 93-100%) if the initial purity of >97% CD3-/CD56+ NK cells was achieved. Viability was >80% directly after harvest from the reactor. Expanded NK cells showed a very strong lysis (median of 42% lysis of K562 cells at E/T ratio of 10:1, range 17-55%) compared to freshly isolated NK cells (median 27%, range 15-41%). Analysis of ADCC demonstrated high lytic activity (median 57%, range 20-70%) towards otherwise resistant lymphoma cells.
Conclusions: Novel GMP-compatible components allow rapid expansion and enrichment of NK cells. The availability of nearly unlimited numbers of activated NK cells with high lytic activity against various tumour targets *in vitro* offers new treatment options for leukemia patients receiving haploidentical transplantats by strengthening of the anti-leukemic effect of the approach.

**P851**

**Irradiated mononuclear cells express significant in-vitro cytotoxic activity. Promise for *in-vivo* clinical trial**

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T cell depletion is necessary in the haploidentical transplantation (HT) setting for prevention of graft *vs* host disease (GvHD). We could hypothesize that repeated infusions of donor NK cells (after T cell removal) could eradicate minimal residual disease (MRD) following transplant without causing GvHD. The removal of T cells in order to give “pure” NK cell infusions is very costly because infusions should be repeated several times. A cheap alternative is to irradiate T cells using a dose of at least 25Gy that has been proved protective against transfusion associated GvHD. The objective of this study was to assess the effect of radiation on the cytotoxicity of NK cells *in vitro*.

Methods: Effector cell (EF) population was mononuclear cells (MNCs) isolated from the peripheral blood of healthy donors. kappa 562 cell line was used as a target (TA) of EF cytotoxicity. Cytotoxicity was assessed by flow cytometry. Target K562 cells were labeled using IgG-FITC. EF and TA were co-cultured for 4h in different EF:TA ratios: 5:1,10:1,20:1,30:1. Cytotoxicity was calculated as follows: specific lysis%=[1–(annexin-negative tau alpha in co-culture/annexin-negative au alpha in control)]X100. A statistical analysis was performed to compare cytotoxicity of fresh MNCs and irradiated MNCs.

Results:

1) Cytotoxicity of fresh MNCs was studied in 13 healthy donors. Mean cytotoxicity in cell cultures with a EF:TA ratio of 5:1,10:1,20:1,30:1 was 61%,65%,70%,78% respectively.

2) The effect of MNC irradiation (25Gy) was evaluated in 6 donors. Cytotoxicity was evaluated in all four types of cultures first using fresh MNCs and then using irradiated MNCs: Cytotoxic activity against K562 was higher when fresh MNCs were used as effectors (p=0.027). Interestingly irradiated MNCs retained significant cytotoxic activity against K562: Cytotoxicity was only diminished by 5-10%.

Conclusion: Irradiated MNCs preserve significant cytotoxicity *in vitro*. It is possible that repeated infusions of large doses of irradiated MNCs in patients undergoing HT could eradicate MRD without a GvHD flare-up. Though irradiated NK cells have a lifespan that is much shorter than that of selected “pure” NK cells, they have the advantage of a much lower cost. We hypothesize that irradiated NK cells could be applied in a clinical trial setting.

**P852**

**Donor lymphocyte infusions for the treatment of chronic myelogenous leukaemia relapse following peripheral blood or bone marrow stem cell transplantation**

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Background: Peripheral blood used as a source of stem cells for transplantation is known to exert stronger immune-mediated effects compared to bone marrow. We decided to analyze the impact of stem cell source on the overall survival (OS) of CML patients who relapsed after alloSCT and had been treated with donor lymphocyte infusions (DLI).

Materials and Methods: We collected data on 384 patients who received DLI after experiencing a CML relapse. These data have been retrospectively retrieved from the EBMT registry. Patients underwent either peripheral blood stem cell (PBSCT, N=168) or bone marrow transplantation (BMT, N=216) from matched family donors after standard intensity conditioning in 1996-2005. All outcomes were calculated from the day of first DLI.

Results: Patients relapsing after PBSCT or BMT differed in several respects including median year of alloSCT (2000 *vs*. 1998), CML phase at alloSCT (77 *vs*. 88% in CP1), and median duration of remission after alloSCT (10 *vs*. 18 months). Univariate analysis revealed a significantly lower probability of OS after DLI in PBSCT *vs*. BMT patients (76% *vs*. 87% at 1 year, 66% *vs*. 79% at 5 years). However, a multivariate Cox analysis for the impact of source, corrected for risk factors at SCT and DLI, did not confirm the significance of PBSCT as a risk factor for decreased OS for the patients transplanted in CP1 (HR 1.036, 95%CI 0.619-1.734). An interaction term suggested that the impact of stem cell source on OS after DLI was different for those transplanted in advanced phases (negative impact of previous PBSCT - HR 2.176, 95%CI 0.930-5.091). The previously observed difference in OS was most likely caused by overlapping of PBSCT with other factors for which significance was confirmed: advanced phase at alloSCT (HR 2.26, 95%CI 1.173-4.354), interval from alloSCT to relapse (HR 0.975 per month difference, 95%CI 0.958-0.993), cytogenetic (HR 3.05 at DLI, 95%CI 1.128-8.222; decreasing effect over time) or hematologic grade of relapse (HR 14.97, 95%CI 6.317-35.474; idem).

Conclusions: The stem cell source does not affect the OS of CML patients who underwent PBSCT in CP1, relapsed and were treated with DLI. However, when the patients were transplanted in advanced phases, previous PBSCT seems to negatively affect OS after DLI, compared to BMT.

**P853**

**Impact of CD3/T regs ratio in donor graft on survival rates in allogeneic peripheral blood stem cell transplantation**

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Introduction: The therapeutic efficacy of allogeneic stem cell transplantation (alloSCT) for hematological malignancies relies largely on the graft versus leukemia (GvL) effect exerted by the donor CD3 cells, but an uncontrolled graft-versus-host-disease (GvHD) bears a risk of complications. On the other hand, T regs cells (CD4+CD25high Foxp3+) are believed to maintain tolerance and to inhibit GvHD after alloSCT; also, the Foxp3 gene encodes a transcription factor that is a key for thymic development, so T regs cells could preserve an optimal microenviroment for the reconstitution of functional immunity after alloSCT.

Patients and Methods: In this study we analyzed the graft CD3+/Tregs ratio (gCD3/Tregs R) and determined its impact on acute GVHD (aGVHD), immunological recovery and survival rates (OS and DFS) after myeloablative alloPBSCT. We analyzed 75 consecutive patients transplanted with unmanipulated peripheral blood stem cells from an HLA identical related donor (n=50) or an HLA identical unrelated donor (n=25); diagnoses were acute myeloid leukaemia (n=62), acute lymphoblastic leukaemia (n=13).The median CD3+ and Tregs dose administered was 238 (range (r): 67-550) and 12,5x$10^6$/Kg (r: 2-21), respectively; the median gCD3/Tregs R was 19 (r: 8-250). Patients were subdivided into a high gCD3/Tregs R (≥36) group (n=31) and a low gCD3/Tregs R (<36) group (n=44).

Results: The incidence of aGVHD (grade II-IV) in the low gCD3/Tregs R (LR) group was lower than in the high gCD3/Tregs R (HR) group (9/44 or 20% *vs* 24/31 or 77%, p<.001). At multivariate logistic regression, gCD3/Tregs R was correlated both with aGVHD (Odds Ratio (OR): 2.60, 95% CI (1.35,4.90), p=.05) and with CMV infection/disease (OR: 2.45, 95% CI (0.8,5.50), p=.05). The relapse rate at 2 years was significantly affected by the number of transplanted T regs (35% *vs* 20% in the L and H

gCD3/Tregs R group, respectively, p=.05); on the contrary, OS was not affected by gCD3/Tregs R.
Conclusions: Taken together, our data may suggest that Tregs content is able to mediate protective effects against aGVHD, but a longer follow-up after alloSCT is needed to understand the real contribution of gCD3/Tregs R on survival rates.

**P854**
**Adoptive immunotherapy with cytokine-induced killer cells generated with a new GMP-grade protocol**
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Background: Cytokine-induced killer (CIK) cells are typically differentiated *in vitro* with interferon (IFN)-gamma and anti-CD3 monoclonal antibodies (mAb), followed by the repeated provision of interleukin (IL)-2. We have previously shown that thymoglobulin (TG) fosters the efficient generation of functional CIK cells, with no concomitant expansion of potentially tumor-suppressive regulatory T cells, when used in combination with interferon-gamma and IL-2 (ITG2 protocol). It is presently unknown whether the infusion of autologous immune effector cells generated with this novel protocol is feasible and safe.
Methods: Five patients with advanced and/or refractory solid tumors (4 cervical carcinoma and 1 melanoma) who failed previous chemotherapy and radiotherapy regimens were enrolled in the present phase I/II study, which was reviewed and approved by the local Ethical Committee. PBMC collected by leukapheresis from consented patients were stimulated under GMP-conditions with IFN-gamma, followed by TG and IL-2. The median time from enrollment into the study to infusion of the expanded CIK cells was 30 days.
Results: The ITG2 protocol allowed the generation of clinically applicable numbers of CIK cells in all patients in the intention-to-treat analysis. The minimum target dose of $1x10^6$ CD3+CD16+CD56+ CIK cells/kg of recipient's bw was obtained in all patients. After 2-3 weeks in culture, a median of $4.65x10^6$ immune effectors/kg of recipient's bw were infused intravenously. One patient with advanced melanoma died from disease progression before receiving the adoptive transfer of CIK cells. In the remaining 4 patients, CIK cells were administered without any measurable toxicity. Challenge with IFN-gamma, TG and IL-2 translated into a remarkable increase in the frequency of CD3+ T cells and CD8+ T cells, whereas NK cells slightly declined in the cultures. Conversely, the frequency of both CD4+ T cells and regulatory T cells did not change after *in vitro* culture compared with baseline, but CD19+ B cells were dramatically reduced at the end of the culture period. From a clinical standpoint, the median survival was 4.5 months (range 1-13) from the first infusion of CIK cells.
Discussion: This phase I/II study highlighted the feasibility and safety of the administration of CIK cells generated with the ITG2 protocol. Whether CIK cells may control disease burden in heavily pre-treated patients with advanced malignancies will be determined in larger clinical trials.

**P855**
**Serum uric acid levels as early biomarker for engraftment in patients undergoing allogeneic haematopoietic cell transplantation**
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Objective: Delayed engraftment and graft rejection after allogeneic hematopoietic cell transplantation (HCT) can result in life-threatening complications during prolonged aplasia. The discrimination between graft failure and delayed engraftment can be challenging. Early biomarkers indicating the incipient recovery of immunocompetent cells could guide clinical decisions. Uric acid (UA) is one of the most important danger signals to the immune system. We analyzed UA serum levels of patients undergoing HCT and – as a control group – patients undergoing induction chemotherapy for leukemia to evaluate the applicability of uric acid as a sensitive biomarker for bone marrow function.
Methods: Serum levels of UA, as well as differential blood counts of 50 consecutive patients undergoing allogeneic HCT were analyzed. As control, we used patients undergoing autologous HCT (n=50) and induction chemotherapy for leukemia (n=50) during a total of 202 cycles of therapy. Groups were balanced for age and sex. In the transplantation groups indications for therapy were ALL (n=7), chronic lymphatic leukemia (n=2), lymphoma (n=28), AML (n=29), chronic myeloid leukemia (n=1), myelodysplastic syndromes (n=7), myeloproliferative disease (n=3), multiple myeloma (n=12), Ewing-sarkoma (n=2), and germinal cell cancer (n=9).
Results: A significant decrease of UA serum levels was observed in all patients (4.7 mg/dl before *vs.* 1.8 mg/dl after therapy). UA levels remained low independently from allopurinol application. During allogeneic HCT, patients had a single re-increase of UA which occurred earlier (day 9.1 *vs.* day 11.5, $p<0.001$) than the detection of first leukocytes (detection limit 50/µl) and was indicative for the incipient rise of leukocytes in the following days. Patients undergoing autologous HCT had a mean of 1.4 increases which occurred concomitantly with increasing leukocytes. Patients in the leukemia group 2.8 increases of UA during aplasia which were hence not indicative for a leukocyte increase. Moreover, in patients suffering from graft failure (n=3), we observed a drop of UA 3 to 4 days before vanishing leukocytes.
Conclusion: UA serum levels can serve as a more sensitive biomarker for incipient bone marrow function of patients undergoing allogeneic HCT as compared to differential blood counts. Changes in UA serum levels could guide clinical decisions towards stimulation of hematopoiesis with G-CSF or towards a second transplantation in case of graft failure.

**P856**
**PRP in treating patients with chronic wounds**
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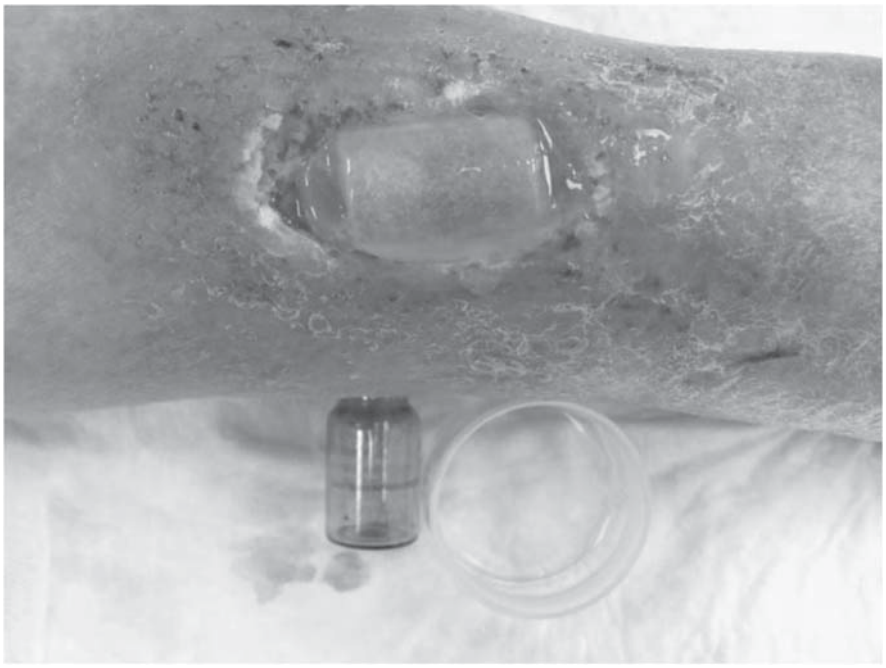
Using platelet-rich plasma (PRP) to accelerate the growth of bone and soft tissues was a breakthrough in dentistry, traumatology, sports medicine, cosmetology, and surgery. This is one of the areas of tissue engineering and cell therapy. There are a variety of publications and studies using a combination of PRP with collagen-containing biologically active agents.
GKB #13 has the experience of PRP in the treatment of chronic nonhealing wounds of various etiologies. Given the presence of wound defects predominantly in soft tissues, PRP is used in the form of a flat gel clot, sometimes in the form of the membrane. Also tested the application of PRP in combination with a collagen preparation "Collost." To obtain PRP we used one-stage centrifugation using a specialized apparatus BTI. Preparation time a bunch of time prior to blood sampling dressing for 20-30 minutes. In 2011 we used the described biologics in the treatment of 38 patients with chronic wounds: one in 24 people a topical treatment was carried out using only PRP, in 5 patients - only "Collost", in 9 patients used a combination of PRP with "Collost." Dressings were performed using both standard and advanced interactive dressings: Tender Wet, Atrauman Ag, Hydrotul, allowing noninvasive cover the membrane from the PRP.
Conclusion: The use of PRP range is wide, as if it were in the form of an independent method, in combination with other biologically active agents that action aimed at stimulation of wound

**[P856]**



healing. It should be noted that all patients after treatment with PRP showed a decrease in pain sensitivity. Choice of treatment with the use of PRP is preferred in patients with chronic non-healing wounds of various etiology and localization, particularly given the ineffectiveness of other methods of treatment. The use of PRP not only reduces the duration and cost of treatment, but also reduces the number of dressings, reduced length of stay of the patient to hospital treatment, as most patients can be observed outpatient, considering the period between dressings in 6-8 days, and also improves the quality of life for patients. The advantages of PRP using: 1. Safer to use autologous materials than allogeneic (homogeneous). 2. Severe soft tissue patronage. 3. Faster mineralization of collagen in the defect area. 4. Growth factors and cytokines fall into the wound, which is impossible, for example, by using a fibrin gel. 5. There is no risk of disease transmission when using autologous blood.

**P857**

**Donor lymphocyte infusions CD3+ after reduced-intensity conditioning allogeneic stem cells transplantation: single-centre experience**

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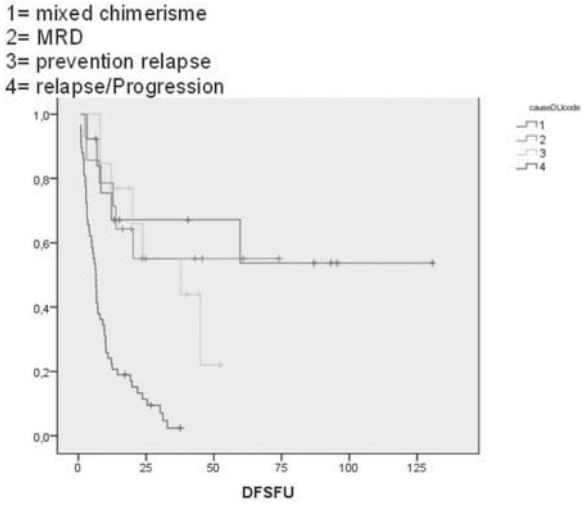
We analyzed 98 patients who received a total of 166 DLI. The median number of DLI was 2 (1-5) the median interval between transplant and DLI was 8 months (1-40), the median number of infused CD3 cells /kg of recipients body weight was $2,8x10^7$ ($1x10^6$-$11,8x10^7$). The diagnoses were multiple myeloma (n=25), acute leukemia (n=19), lymphoma (n=18), chronic myeloid leukemia (n=5), myelofibrosis (n=3), myelodysplastic syndrome (n=2), solid tumors (n=21), other diagnosis (n=5). Median patient age was 47 years (20-67). The cause of DLI was the presence of relapse or progression in 54 patients (55%), residual disease in 17 patients (17%), prophylactic in 14 patients (14%), and the presence of mixed chimerism in 13 patients (13%). Factors studied for an association with GvHD and TRM were donor type(siblings/alternative donors), year of DLI (</>2006), maximum dose of DLI (</>$1x10^7$), recipient age (≤/>50 years), number of DLI (</>1), interval transplant-DLI (</>6 months), cause of DLI (relapse *vs.* prophylaxis *vs.* mixed chimerism *vs.* residual disease), disease type and recipient gender. Eleven patients (11%) developed acute GvHD grade II-IV, and 7 patients (7%) developed an extensive chronic GvHD. In univariate analysis we could identify the interval transplant-DLI <6 months, the cause of DLI and DLI number as a predictors factors of acute GvHD: In multivariate analysis this results was confirmed only for the interval transplant-DLI with HR=0.11 (0.03-0.41) (p=0.001). With a median follow up of 34 months (1-132), 43 patients are alive (44%). The primary cause of death was relapse of the original disease in 50 patients (51%), whereas 4 died of TRM (4%). No factors predicting TRM in univariate analysis were indentified. The overall survival (OS) at 5 years was 45% (34-56)]; the progression free survival (PFS) at 3 and 5 years was 24% (13-33)] and 17% (8-26)] respectively.In this relatively large series of consecutive DLI, the risk of GvHD was relatively low, and we could identify only the interval transplant-DLI less than 6 months as significant predictor of acute GvHD. At a preliminary analisis, DLI



seem to show efficacy when administered as prophylaxis, for mixed chimerism and residual disease but not for overt relapse. Our findings indicate that this form of adoptive immunotherapy is well tolerated and induces a low incidence of GvHD, supporting further investigation as an upfront modality to enhance graft versus tumor response in high risk patients.

**P858**

**Conventional and G-CSF primed DLI: a single-centre experience**

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Background: Relapse is the first aetiology of death after allogenic stem cell transplantation (HSCT). Donor Lymphocyte Infusion (DLI) is a therapeutic approach based on the Graft versus Tumor (GvT) effect. G-CSF primed DLI is an option which has recently been reported as safe and effective.

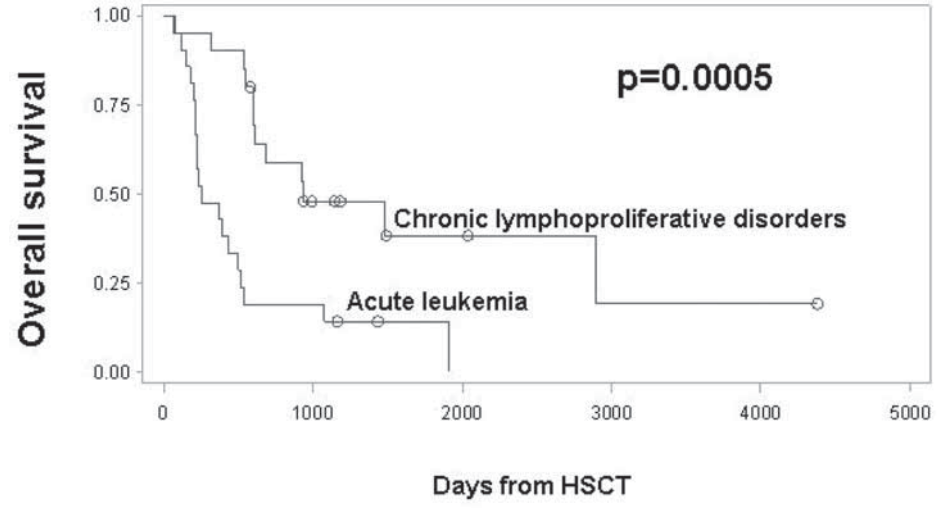
The aim of the study was to compare the outcome of patients who received conventionnal DLI to those who had G-CSF primed DLI.

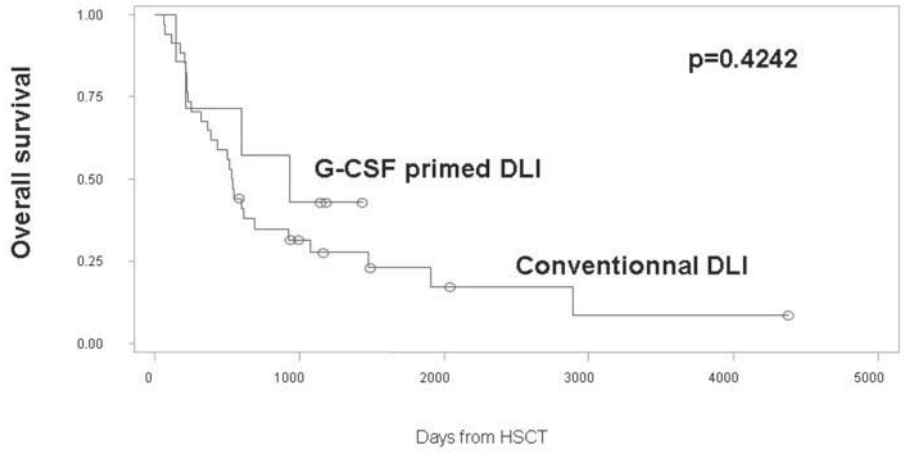
Patients and Methods: We retrospectively analyzed the data of patients who received DLI in our institution from 1998 to 2010.

Results: A total of 55 patients with a median age of 46 (2-67) received a median of 1 DLI (1-6). The two main indications for HSCT were acute leukemia (53%) and multiple myeloma (27%) with a statistically significant better outcome for chronic lymphoproliferative disorders (Figure 1). Indications for DLI were hematologic relapse (71%), infra-hematologic relapse (15%) and mixed chimerism (14%). 42 patients received conventional DLI and 13 had G-CSF primed DLI as first DLI with a median of $10^8$ CD3+ cells/kg at average of 451 days post-HSCT in both group. Between these 2 groups, there was no statistical difference in term of response before and after DLI, Graft versus Host Disease (GvHD) and infections post-DLI. 4 patients died because of GvHD or infections post-DLI, among them, one had G-CSF primed DLI. With a median follow-up of 369 days after the first DLI (692 post-HSCT), median survival was respectively 535 and 938 days post-HSCT for patients receiving conventionnal and G-CSF primed DLI (p=0.4242) suggesting a trend to a better outcome (Figure 2).

In multivariate analysis, the only factors reaching statistical significance are the status of disease prior DLI and the type of haematologic malignancies (lymphoproliferative disease versus acute leukemia).

Conclusion: G-CSF primed DLI are safe and effective but further investigations are needed to assess its potential benefit compared to conventional DLI. Otherwise, results seem better in chronic lymphoproliferative disorders and preclude immunotherapeutic management following HSCT in those patients.



**P859**

**Granulocyte transfusion in paediatric oncology patients with febrile neutropenia**

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Objective: To describe the clinical course of neutropenic pediatric oncology patients undergoing granulocyte transfusions (GTF)
Design: Retrospective chart review including all children receiving GTFs between Sepember 1999 and September 2011.
Setting: University Hospital
Patients: Thirty seven pediatric oncology patients with febrile neutropenia and proven or suspected serious infection.
Interventions: These 37 patients received a total of 51 courses of GTFs
Measurements and Main Results: WBC count was assessed before and 2h and 24h post transfusion. In thirty five courses of GTFs patients had leukemia and in sixteen courses of GTFs patients had solid tumors. 80.4% (n=41) of the patients were not in remission (new diagnosis, relapse, refractory to treatment). Mean duration of the fever and neutropenia was 18±17days. The indications for granulocyte transfusion were bacterial infection (43%, n=22), fever of unknown origin (41.2%, n=21), fungal infection (9.8%, n=5), both bacterial and fungal infection (2%, n=1), antibiotic resistant bacteremia (2%, n=1) and prophylactic (2%, n=1). Granulocyte yield was 3,1±1,2 x1010 and mean WBC count before, 2h and 24h after transfusion were 465±522 $mm^3$, 4411±6709 $mm^3$, 3525±4022 $mm^3$ respectively. GTFs were well tolerated except one patient who had fever during transfusion. Thirty nine (76.5%) of the courses resolved from infection and discharged from the hospital. Of the twelve courses (23.5%) who died, seven of them were refractory to treatment, two of them were new diagnosis and three of them had relapsed disease.
Conclusions: This case series documents the course of 37 septic neutropenic pediatric oncology patients who underwent a total of 51 GTF courses. GTFs were generally well tolerated and improve short term outcome in neutropenic pediatric oncology patients.

**P860**

***In vitro* expansion of human natural killer cells under good manufacturing practice conditions for multiple mega dose infusions as immunotherapy of haematopoietic malignancies**

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Acute myeloid leukemias are blood cell malignancies with significant relapse rates despite currently available treatments with high dose chemotherapy and allogeneic stem cells transplantation (HSCT). Post transplantation donor lymphocyte infusions (DLI) with natural killer (NK) cells represent a novel strategy to enhance the graft-versus-leukemia effect of the treatment. We previously described the feasibility of purifying peripheral blood NK cells in numbers sufficient for a single infusion of up to 3.3x10e7 cells/kg body weight (BW). According to experimental animal data, to achieve clinically relevant effector to target ratios *in vivo*, particularly in patients with partial remission and persistent tumor burden, multiple infusions of mega doses of cytokine-activated NK cells with minimal T cell contamination are needed. Here we describe the first clinical expansion of NK cells at the good manufacturing practice (GMP) facility of the Hematology Clinic, University Hospital Basel for multiple NK-DLI after haploidentical HSCT.
NK cells were purified from 8.9L unstimulated leukapheresis of a haploidentical stem cell donor by T cell depletion and subsequent NK cells selection using CliniMACS. Starting from 119x10e8 total nucleated cells, 4.3x10e8 NK cells were obtained with a purity of 94.9% and a residual T cell contamination of 0.63x10e6 cells corresponding to a T cell depletion efficacy of 4.07 log. Purified NK cells were expanded using our recently described protocol. Cells were cultured in air-permeable bags in up to 11.5L medium containing human serum, IL-2, IL-15, anti-CD3 monoclonal antibody and irradiated autologous feeder cells. After 19 days NK cell numbers increased 74-fold, i.e. 301x10e8 cells with a viability of 78%. CD3+ T cells concomitantly expanded to 43x10e6 cells. A second CliniMACS T cell depletion was performed to decrease the T cell number below the limit of 0.5x10e5 cells/kg BW of the recipient. T cell content was reduced by 2.06 log to 0.1x10e5 cells/kg BW, however with a significant NK cell loss of 21%. The NK cell product was divided and cryopreserved as 6 escalating doses ranging from 1.0x10e6 to maximally 1.0x10e8 cells/kg BW. All 6 NK-DLIs given within 30 days of haploidentical HSCT were tolerated without any acute adverse effects. These results demonstrate the feasibility of large-scale expansion under GMP conditions and multiple mega dose infusions of human NK cells as immunotherapy after allogeneic HSCT for malignant disease.

**P861**

**GMP compliant generation of donor-derived peptide-stimulated CMV and EBV specific T-cells derived from G-CSF mobilised stem cell grafts**

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The reactivation of CMV and EBV after allogeneic stem cell transplantation (SCT) impacts negatively on outcomes. Following allogeneic SCT approximately 20-30% of all patients reactivate EBV and 40-50% CMV, respectively. Specific antiviral therapy is only available for CMV. With the exception of Ganciclovir all drugs are being used outside their approved indication. Similarly, the use of Rituximab as B-cell depleting antibody seems to be effective in case of EBV reactivation, but bears strong side effects including long term B-cell depletion making frequent transfusion of immunoglobulins a must. Furthermore, all antiviral therapies are cost-intensive.
In this study we developed a manufacturing protocol according to GMP standards that allows the generation and expansion of T-cells with specificity for CMV and EBV out of the G-CSF mobilized stem cell graft from EBV and CMV seropositive donors. G-CSF mobilized PBMC were purified using Ficoll gradient centrifugation and stored in liquid nitrogen for generation of T-cells after engraftment had occurred. After thawing, up to 1x109 PMBC were stimulated with 21 peptides derived from CMV and 29 peptides derived from EBV. All peptides exhibit a defined HLA restriction, which covers 80% of haplotypes within the central European population. After peptide stimulation cells were transferred into closed system culture bags and incubated for 9 days at 37°C, 5% $CO_2$ in GMP certified media supplemented with 50IU/ml IL-2. Reactivity of PBMC for both viruses prior to peptide stimulation ranged from 0.1% to 0.7%, as tested by ELISpot; after incubation on average 15% of cells displayed reactivity against EBV and CMV, resulting in an up to 100-fold expansion. Flow cytometric analyses by pentamer staining confirmed ELISpot results showing up to 60% specificity and an activated phenotype. Cells could be stored in liquid nitrogen and remained stable for more than 100 days by to date.
In summary, this protocol exhibits several advantages: (i) Donor safety: the stem cell graft can be used as a source for PBMC, no second apheresis is required. (ii) Timing: PBMC as raw material can be stored stably until generation of T-cells is desired. (iii) The manufacturing process makes use of standard clean room equipment. (iv) The protocol can

be easily adapted to local requirements and preferences. (v) Costs for production would be competitively low compared to current prices for antiviral therapies and associated complications.

**P862**

**T-cell depletion in allogeneic stem cell transplantation for myelofibrosis: the Swiss experience**

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Objective: Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment in myelofibrosis (MF). We report a retrospective analysis on the impact of T-cell depletion (TDEP) in prophylaxis of GvHD on the outcome of HSCT in MF.
Methods: Between 06.1997 and 05.2011 35 patients (24 M/11 F) with a median age of 55 years (range 25-69) underwent HSCT from an identical sibling donor (51%), a MUD or MMUD (43%) or an alternative donor (6%). At transplant most patients had an intermediate-2 DIPPS, 31% had a prior splenectomy and 40% had a JAK2V617F mutation. 18 patients received a RIC regimen mostly fludarabine-based while 17 patients received a myeloablative regimen with BU-CY (71%) or CY-TBI±VP16. *Ex vivo* TDEP (Campath in the bag) was used in 11 patients, *in vivo* TDEP (ATG) in 13 and no TDEP in 11. GvHD prophylaxis included CSA ± MTX or MMF.
Results: Thirty-two patients engrafted. Two patients in the *in vitro* TDEP group did not engraft and died from infection. Seven patients died from relapse and 5 from non-relapse mortality (NRM). A total of 21 (60%) patients developed acute GvHD grades I-IV (grades II-IV in 28%). There was significantly less acute GvHD after *in vitro* TDEP (p=0.01) (due to decreased acute GVHD grade I in this group). Chronic GvHD developed in 21 patients, without statistical difference according to the TDEP status. 7 of the 13 patients who relapsed were in the *in vitro* TDEP group. 3 were successfully treated with donor lymphocyte infusion (DLI) and 3 additional patients receiving DLI because of mixed chimerism achieved a long term remission. Hematological remission and histological response was achieved in 80% of patients. There was no difference in full donor chimerism incidence or in the time to engraftment between the TDEP groups. With a median follow-up of 36 months (range 3-420), 3-years overall survival (OS) and current disease-free survival (cDFS) were both 68% (95% CI 52-84%), 3-years NRM was 20% (95% CI 4-36%) and relapse incidence was 25% (95% CI 7-43%). OS did not differ according to TDEP (p=0.30), stem cells source (p=0.40), type of conditioning (p=0.99), JAK2 mutation (p=0.47) or prior splenectomy (p=0.57); there was a tendency of lower incidence of relapse in the *in vivo* TDEP group (p=0.05).
Conclusion: A significant proportion of patients with MF achieved long-term survival after HSCT. *In vitro* TDEP was associated with increased incidence of relapse. DLI was an effective salvage therapy for many of those patients.

**P863**

**Prognostic significance of EBMT risk score for the outcome of allogeneic haematopoietic stem cell transplantations after T-cell depletion**

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Objective: We investigated whether the EBMT risk score, which is broadly predictive of the outcome after hematopoietic stem cell transplantation (HSCT) is also valid in our center, where 73% of patients are transplanted with T-cell depleted grafts (TDEP).
Methods: We analyzed 513 patients with various hematological diseases transplanted between 1984 and February 2011 with grafts from identical siblings (312, 61%), MUD (121, 25%), MMUD (21, 4%) or alternative donors (59, 12%). The median follow up for alive patients was 7 yrs (range 0.5-27 yrs). The median age of patients (368 M, 145 F) was 38 years (range 1-70). TDEP was with CAMPATH (1M,1G or 1H).Stem cell source was bone marrow (35%), peripheral blood stem cells (64%) or cord blood (1%). Ninety-one patients received a fludarabine based reduced intensity conditioning (RIC) regimen and 422 patients a myeloablative regimen (MAC) with BU-CY (17%) or CY-TBI±VP16 (83%).GvHD prophylaxis consisted of CSA±MTX or MMF.
Results: OS at 5 years was 51±4%, the incidence of relapse, relapse death or transplant-related mortality (TRM) were 42±5%, 26±4% and 26±4% respectively. OS at 5 years of patients with risk score 0/1 was 79±11% and 44±24% for patients with risk score 6/7 (p<0.0001). Patients with a higher EBMT risk score had a higher incidence of relapse and relapse death (p<0.0001) and more TRM (38±26 *vs* 14±10%), but this was not statistically significant (p=0.14). Patients receiving RIC, who had higher risk scores than patients after MAC (p<0.0001) had a higher rate of relapse (52±12% *vs* 33±5%, p<0.0001) and relapse death (45±12% *vs* 24±5%, p<0.0001) and a lower OS (43±11 *vs* 58±5% p=0.044), but, the EBMT score on OS, TRM, relapse and relapse death of patients after MAC or RIC was similar. The EBMT score affected OS, relapse rate and relapse death in recipients of T cell depleted/replete grafts comparably but had only a marginal impact (p=0.042) on TRM in patients after TDEP and no impact in patients receiving unmanipulated grafts.
Conclusion: Our study confirms the prognostic value of the EBMT risk score on OS and relapse rates. By contrast, we only found a marginal impact on TRM. Hence, the EBMT risk score represents a simple and reliable tool for systematic and standardized pretransplant risk assessment. Nevertheless, risk assessment for an individual patient remains complex, while outcome depends not only on the pretransplant parameters of the EBMT score but also on many peritransplant and post-transplant factors.

**P864**

**Prophylaxis and pre-emptive donor lymphocyte infusions in patients with high risk for disease relapse after allogeneic haemapoietic stem cell transplantation**

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Objectives: To evaluate the efficacy and toxicity of preemptive and prophylaxis donor lymphocyte infusions (DLI) in patients (pts) with hematological malignances who are at high risk for relapse after allogeneic hemapoietic stem cell transplantation (allo-HSCT).
Methods: In this study we retrospectively analyzed preemptive and prohylaxis DLI in 28 patients who underwent allo-HSCT for ALL (n=12), AML (n=6), acute biphenotypic leukemia (n=2), CML (n=5), and MDS (n=3). The median pt age was 21.9 (range 1–53) years. At the time of allo-HSCT 11 pts had CR, 7 pts had minimal residual disease (MRD), and 10 were in relapse or an acceleration phase. Allo-HSCT were performed from matched related (n=9), matched unrelated (n=14), and haploidentical (n=5) donors. Ten and 18 patients received myeloablative and reduced intensity conditioning respectively. The indications for DLI were MRD in 11 pts (MRD was detected by cytogenetic method, PCR or flow-cytometry), falling chimerism in 8 pts, and disease relapse prophylaxis in 9 pts in the high-risk group.

Thirteen pts received DLI in combination with tyrosine kinase inhibitors, IFN-gamma, IL-2, GM-CSF, and 15 pts received DLI alone. Fifteen pts received DLI by a bulk dose regimen (total cell dose (TCD) ranged from $1X10^4$ to $8X10^7$ CD3+/kg), and 13 an escalating dose regimen (TCD ranged from $2X10^5$ to $1.2X10^8$ CD3+/kg).
Results: Eight (72%) pts with MRD achieved MRD-negative status after DLI, and 1 pt relapsed 12 months after DLI. Full donor chimerism was achieved in 4 pts (50%). The duration of CR in pts who received prophylactic DLI was 6–16 months, and 2 pts developed disease relapse. Acute graft versus host disease (GVHD) grade III–IV occurred in 3 pts (11%), and in 2 pts it was fatal. Chronic GVHD occurred in 11 pts. Four-yr OS was 73%, and DFS was 78%. At the time of follow-up 21 pts are alive, including 18 in CR.
Conclusion: We conclude that preemptive and prophylaxis DLI is feasible in patients who are at high risk for relapse after allo-HSCT. However, this method may be associated with severe acute GVHD, and need further investigation.

**P865**
**Haploidentical DLI for relapse post haplo bone marrow transplantation with high- dose of cyclophosphamide post-transplant as GvHD prophylaxis: is it feasible?**
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Background: The use of donor lymphocyte infusions (DLI) from HLA mismatched donors, is associated with a high risk of acute GvHD. In the Perugia studies the threshold is set at $10^3$ CD3/kg. In one study (Huang X, BBMT 2009) a median T-cell dose of $2.4x10^8$/kg (from mobilized PBSC) was administered to recipients of haploidentical stem cell transplantation : the cumulative incidence of chronic GVHD exceeded 50%.
Aim of the Study: We tested the feasibility and occurrence of GVHD following 12 haploidentical DLI in seven patients, who relapsed after unmanipulated haploidentical T-cell replete BMT, without additional immunosuppression.
Patients and Methods: All patients were transplanted from haploidentical related donor after myeloablative conditioning (n=3) or non myeloablative conditioning (n=4), and received high-dose of CY post-transplant as GVHD prophylaxis, in association with cyclosporin and micophenolate. The diagnosis was Hodgkin's lymphoma (n=3), acute lymphoblastic leukemia (n=2), acute myeloid leukemia (n=1), chronic myeloid leukemia blast crisis (n=1). The median time of relapse was 200 days from BMT (range 45-350 days). DLI were collected by apheresis from the haploidentical donor. The median time of first infusion was 72 days from relapse (range 28-342 days) and 235 days from BMT (range 119-519). At time of first DLI all patients were off GVHD prophylaxis. The minimum dose of CD3+ cells was $1x10^3$/kg; two patients received two DLIs, with dose escalation of half log in the second administration, another patient received four DLIs, at the dose of $1x10^5$/kg and $1x10^6$/kg respectively. In all cases DLI were given after a course of chemotherapy. Median time of DLI was 12 days from chemotherapy (range 10-14 days). Chimerism was 100% donor in all patients at time of first DLI.
Results: The infusions were well tolerated and no major adverse effect were observed. No patient developed GVHD; no aplasia related to DLI occurred. Two patients died with progressive disease; five patients are alive, three of them are disease-free. The median follow-up from the last DLI was 125 days (range 40-342 days).
Conclusions: These data suggest that HLA haploidentical DLI can be administrated at doses ranging from $1x10^3$/kg to $1x10^6$/kg. Further exploration of this therapeutic approach seems warranted.

**P866**
**Minimally-manipulated cord blood regulatory T-cells consistently suppress target cell responses under appropriate conditions**
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Objectives: Regulatory T cells (Tregs) are increasingly being considered as tools to induce tolerance post-transplantation, with recent clinical trials of adult Tregs and Tregs lines from cord blood (CB) being performed. Since only expanded cord Treg lines have currently been used in this context, we focused, in a pre-clinical study, on ascertaining if minimally manipulated CB Tregs can be a source for therapy; comparing the conditions that allow for suppression by both CB or adult Tregs.
Methods: The function of freshly isolated cord Tregs (using the CD25 CliniMACS reagent; Milteni Biotec), to polyclonal stimuli (1µg/ml soluble anti-CD3 and 2µg/ml soluble anti-CD28 antibodies) were assessed and compared with an equivalent population of adult Tregs, to determine if there are conditional requirements for full cord Treg functionality ex-vivo.
Results: CB effector cell (CD4+CD25-; Teff) responses to anti-CD3/28 were weak compared to adult cells and, unlike adult, with no maximal response observed in a cell dose response curve from combined data. Under these conditions, only adult Tregs demonstrated consistent suppression. However, in the presence of exogenous IL2 (800 IU/ml), CB Tregs achieved a cell dose response curve similar to that seen with adult and demonstrated suppression (6 CB, 4 adult). With both adult and CB, it was found that an optimal Teff cell dose/response existed at which Tregs demonstrated the highest levels of inhibition (at a fixed Teff:Treg ratio of 3:1). At these optimal conditions ($1x10^5$ Teff, 800 IU/ml IL-2 and anti-CD3/28) suppression of adult Teff by CB Tregs and CB Teff by adult Tregs was similarly observed.
Conclusion: These data indicate that the effector dose/response is an important factor in determining suppression. Moreover, when assessing suppression to a particular stimulus (especially alloreactivity and antigen responses), the response of the target cells needs to be considered. In optimal conditions cord Tregs can be functional against cord and adult Teff and if target cell responses are strong then cord Tregs should be functional without extensive manipulation making these cells attractive for therapy.

**P867**
**Haematopoiesis-specific T-cells may induce GvHD via induction of collateral damage to non-haematopoietic cells**
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Donor T cells directed against hematopoiesis-restricted minor histocompatibility antigens (MiHA) are assumed to give rise to a specific graft-versus-leukemia (GvL) effect without graft-versus-host-disease (GvHD) following allogeneic stem cell transplantation (alloSCT). However, It has been observed that overt GvL responses, associated with occurrence of T-cells directed against hematopoiesis-restricted MiHA can coincide with limited GvHD. To analyze whether T cells mediating a specific GvL response may under certain conditions induce damage to surrounding non-hematopoietic tissues, we stimulated MiHA-specific T cells with MiHA-positive hematopoietic targets on a monolayer of MiHA-negative primary human fibroblasts seeded in the same well. We demonstrated that CD4 and CD8 MiHA-specific T cells activated by MiHA-positive hematopoietic stimulator cells (EBV-LCL/DCs), induced 60-100% bystander cytotoxicity to the surrounding fibroblasts at a 5/1 T-cell/fibroblast-ratio. T-cell activation by the hematopoietic cells was pivotal for the induction of this collateral damage. We demonstrated

that increased strength of T-cell activation correlated with increased severity of collateral damage. Using T-cell activation in a cell free (PHA or antiCD3/CD28 beads), we illustrated that the MiHA-negative fibroblasts are not attacked due to cross-presentation of HLA/peptide complexes from apoptotic hematopoietic cells. Next, we investigated the prerequisites for collateral damage induction to occur. Using a transwell system, we demonstrated that direct T-cell-fibroblast interaction was required. By inhibition of the death receptor-, mitochondrial-, or granzyme-B (GzB)-mediated apoptosis induction pathways via retroviral transduction of the anti-apoptotic proteins c-FLIP, Bcl-2 or PI-9 in fibroblasts and inhibition of T cell degranulation with EGTA, we demonstrated that the main effector mechanism underlying collateral damage is release of GzB- and perforin-containing granules by T-cells upon activation. Misdirection of release of cytotoxic granules towards the MiHA-negative non-hematopoietic targets may underlie the induction of collateral damage. In conclusion, these data suggest that hematopoiesis-restricted T-cells actively participating in an overt GvL response can induce GvHD by inducing collateral damage to MiHA-negative non-hematopoietic targets via misdirection of their cytotoxic execution machinery when they are in close proximity of the non-hematopoietic tissues.

**P868**

**Allogeneic cytokine-induced killer cells kill dendritic cells efficiently, resulting in less graft-versus-host disease**

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Cytokine-induced killer (CIK) cells are *ex vivo*–expanded T lymphocytes expressing both natural killer (NK)– and T-cell markers. We have reported that adoptive transfer of allogeneic CIK cells in a murine model caused minimal graft-versus-host disease (GVHD) with retention of antitumor activity mediated by NKG2D, which is an activating receptor expressed on NK cells. The mechanism of suppression of GVHD after allogeneic bone marrow transplantation is, in part, due to the abundant production of IFN-gamma from the CIK cells, which has protective effect against GVHD. We have also demonstrated that allogeneic CIK cells displayed a significant lower acquisition of homing molecules, required for the entry of inflamed and GVHD target organs and a higher susceptibility to apoptosis compared to allogeneic splenocytes. There also might be some causes other than we mentioned above. Host residual dendritic cells (DCs) have a crucial role for initiating GVHD reaction, because they present recipient alloantigens to donor T cells, which finally attack on recipient tissues. Murine alloreactive NK cells, even when infused in large numbers, do not cause GVHD in the mouse by killing recipient DCs. Similarly, it remains a possibility that the reduced GVHD in CIK cells receiving mice was due to the elimination of residual host DCs by CIK cells. To test this, DCs generated from bone marrow cells were used for 51-Cr release cytotoxicity assays as target cells. Although autologous CIK cells (Balb/c) had relatively strong killing activity against DCs (Balb/c), allogeneic CIK cells (B6) had much more killing activity even from at a 5:1 effector -target ratio. Allogeneic CD8 positive cells did not show any killing activity against DCs. In addition, killing activity against DCs did not changed with/without adding NKG2D blocking antibody, suggesting that other mechanisms to undergo cell lysis should exist in CIK cells in addition to NKG2D/NKG2D ligand system. To further evaluate whether allogeneic CIK cells kill host DCs *in vivo*, lethally irradiated Balb/c recipients were given BM (B6) with CIK cells or splenocytes to compare the percent of residual host-typed DCs in the spleen five days after bone marrow transplantation. Percent of host-typed DCs tended to be lower in the mice receiving alllogeneic CIK cells compared with those receiving fresh splenocytes or BM alone. In conclusion, allogeneic CIK cells caused less GVHD due in part to elimination of host DCs.

**P869**

**Development of antibody responses against H-Y antigens and their X-variants after allogeneic haematopoietic stem cell transplantation and donor lymphocyte infusion**

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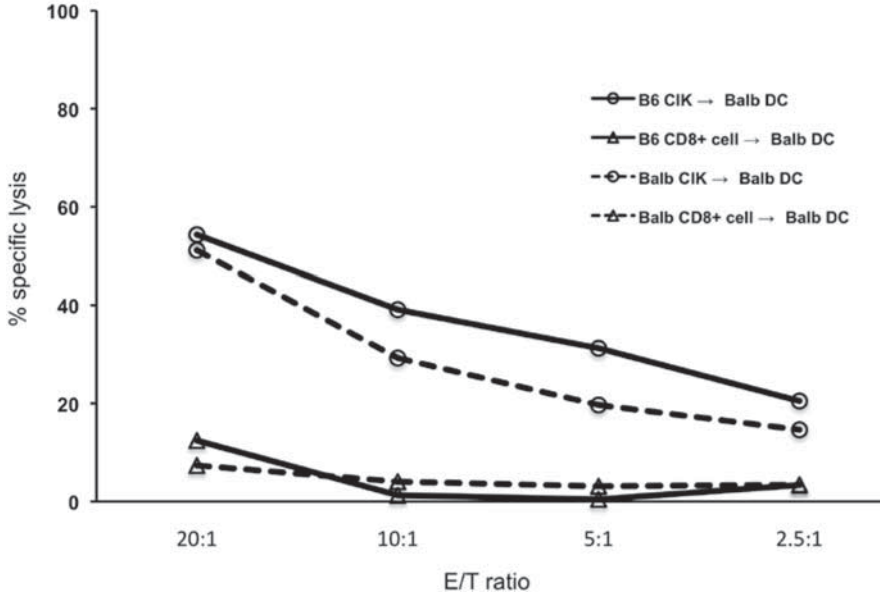
Introduction: Allogeneic stem cell transplantation (alloSCT) is an effective treatment for patients with hematological malignancies. At the Leiden University Medical Center, T-cells are

**[P868]**

Fig.1 CIK cells have strong cytotoxicity against DCs



depleted from the stem cell graft to reduce the incidence and severity of graft-versus-host disease (GvHD). Donor T-cells are re-administered later as donor lymphocyte infusions (DLI) to preserve graft-versus-leukemia (GvL) reactivity. Various studies have demonstrated that not only T-cells, but also antibodies are induced after alloSCT. The objective of this study was to evaluate whether antibodies can be used to follow clinical responses after treatment with alloSCT and DLI.
Methods: We developed a Luminex bead assay to measure antibodies against multiple antigens encoded by the Y-chromosome (H-Y antigens) and their respective X-variants in small sample volumes. RPS4Y, ZFY and DBY and their X-variants were produced as overlapping protein fragments in E Coli. The protein fragments were coupled on Luminex beads, and 400 serum/plasma samples from 43 patients with chronic myeloid leukemia collected before and during treatment with alloSCT and DLI were screened for antibody binding to the antigen-coupled beads.
Results: Antibodies against H-Y were measured in 3/16 female and 9/27 male patients. ZFY was most frequently targeted (9x), but (simultaneous) antibodies against DBY (5x) and RPS4Y (6x) were also found. Antibodies against H-Y were most frequently observed in female-to-male (6/9) as compared to male-to-male (3/18), male-to-female (2/10) and female-to-female (1/6) transplantations, and were often induced after alloSCT or DLI. Notably, all male patients transplanted with female donors with treatment-induced antibodies against H-Y had also detectable antibodies against X-variants.
Conclusions: Antibodies against multiple protein targets can be successfully measured in small sample volumes by a Luminex bead assay. Strong humoral responses against H-Y were measured most frequently in male patients transplanted with female donors after DLI. Our data therefore show that antibodies can be used to follow clinical responses after alloSCT and DLI. The data also suggest that antibodies against ubiquitously expressed intracellular H-Y antigens develop as a result of cellular debris induced by treatment with alloSCT and DLI, and that these antibodies often display cross-reactivity towards X-encoded homologues.

**P870**

**HLA-DP antigens are major targets of AML-reactive CD4+ T-cells isolated from HLA-DR/DQ matched donors *in vitro***

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The graft-versus-leukaemia (GvL) effect after allogeneic haematopoietic stem cell transplantation is mainly mediated by T cells of donor origin that recognize HLA-associated antigens on patient-derived leukaemia cells. Previous research has mainly focused on CD8+ cytotoxic T lymphocytes representing a major part of GvL effector cells. However, CD4+ T cells, commonly regarded as helper cells of the adaptive immune system, are also capable of executing direct cytolytic activity. This as well as the virtual absence of HLA class II expression on non-haematopoietic cells under non-inflammatory conditions makes CD4+ T cells also attractive mediators of the GvL response. Here we sorted phenotypically naive CD4+ T cells from healthy donor PBMC by immunomagnetic or flow cytometry means and stimulated them against primary acute myeloid leukaemia (AML) blasts matched for HLA-DR and –DQ but not –DP alleles, reflecting the common clinical situation. AML-reactive CD4+ T-cell populations were analysed for cytokine secretion, HLA restriction, and T cell receptor V-beta chain usage. Their ability to lyse target cells was measured in 5h chromium-release assays. In 3 extensively studied donor/patient models, leukaemia-reactive CD4+ T-cell clones could be expanded that were mainly restricted by HLA-DP alleles according to antibody blocking experiments. T cells showed functional properties of Th1 type cells, producing IFN-gamma, TNF-alpha, but not IL-4 upon AML stimulation. They lysed primary AML blasts as well as patient-derived EBV-B cells at moderate to strong levels (i.e. up to 100% at effector/target ratio of 60/1). Moreover, CD4+ T cell clones cross-reacted with AML blasts derived from other patients expressing the same single HLA-DP mismatch allele as patient cells used for initial stimulation, suggesting that these T cells are directed against disparate HLA-DP alleles.
In summary, we demonstrate herein that AML-reactive CD4+ T cells can be reliably isolated and expanded from naive precursors of HLA-DR and –DQ matched healthy donors. Most of them appear to recognize HLA-DP mismatch alleles, indicating a potentially important role of these alleles as targets of the GvL response.

**P871**

**Influence of IFNgamma on induction of IDO in a human *in vitro* skin graft-versus-host disease model**

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In Graft-versus-Host Disease(GvHD) T cell activation leads to a cytokine storm involving IFNgamma(IFNg) and TNFalpha. Although IFNg is a typical inflammatory cytokine, it is also discussed to have immunomodulatory effects by induction of indoleamine2,3-dioxygenase(IDO) the intracellular enzyme converting tryptophan(Trp) to kynurenine catabolites. Kynurenines(Kyn) exert immunosuppressive functions by suppression of effector T cells and generation of regulatory T cells. To address the question whether IFNg treatment can influence GvHD via induction of IDO and its metabolites we used a human *in vitro* model of skin GvHD–the skin explant assay (SE).
In brief, first a mismatched(mm) MLR was set up for 7 days with responder cells of the donor and irradiated patient cells. After further three days of co-culture of recipient skin and MLR the skin explants were histopathologically graded according to Lerner. In different set-ups MLR or skin were incubated with different concentrations of IFNg. Induction of IDO was assessed by measurement of Kyn in tissue culture supernatants by tandem-mass spectrometry. Kyn concentrations were correlated with GvHD stage. To check the endogenous contribution of IDO we incubated the SE with the IDO-inhibitor 1-methyl-L-tryptophan (1-MT).
In SE we observed similar values of Trp and Kyn catabolites for medium and MLR control.IFNg increased Kyn, anthranilic acid and 3-OH-anthranilic acid in a concentration dependent way in MLR alone, skin alone and the SE. 1-MT resulted in inhibition of Kyn synthesis in MLR and SE only in the presence of IFNg. Pre-incubation of the skin with IFNg for different intervals before adding the MLR had no effect on Kyn production. There was neither improvement nor deterioration of the histopathological GvHD grading during IFNg treatment but damage in the mm MLR control was already at highest level.
Our experiments show that IFNg leads to *in vitro* induction of IDO both in activated peripheral blood cells as well as in cutaneous cells during alloreaction. The failure to improve histopathological GvHD grading and the lack of 1-MT activity in the absence of IFNg suggest a minor role of IDO in the baseline alloreaction at least in the model of the mm skin explant assay. Further experiments including qRT-PCR for IDO are planned to clarify the exact contribution of the IDO pathway. Our data may contribute to explain why clinical use of IFNg fails to exacerbate GvHD and provide additional insights in its immunomodulatory mechanisms.

**P872**

**Comparison of two formulations of filgrastim, Neupogen (Amgen) and Zarzio (Sandoz), used to accelerate neutrophil recovery after autologous peripheral blood stem cell transplantation**

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Granulocyte-colony stimulating factors, including filgrastim are widely used to support neutrophil recovery after autologous peripheral blood stem cell transplantation (autoPBSCT). In recent years biosimilars of originally developed filgrastim became available and are postulated to provide the same safety and efficacy profile. The goal of the current study was to compare for the first time Neupogen (Amgen, original formulation) with Zarzio (Sandoz, biosimilar) in a setting of autoPBSCT.

35 consecutive patients with multiple myeloma (MM) were treated with Neupogen between 04.2010-05.2011 while 55 patients received Zarzio between 06.2011-11.2011. Filgrastim 5 ug/kg was administered since day +7 until neutrophil recovery >0.5 x10e9/L for two consecutive days. Median age was 59 (47-66) years in the Neupogen group and 57 (47-69) years in the Zarzio group. The proportion of first and second of the planned tandem transplantations was 29(83%)/6(17%) for Neupogen and 31(56%)/24(44%) for Zarzio. The number of transplanted CD34+ cells was significantly higher in the Zarzio compared to Neupogen group (6.7±3 x10e6/kg *vs.* 3.9 ±3 x10e6/kg, respectively). Patients were administered a uniform anti-infectious prophylaxis consisting of ciprofloxacin and fluconasole.

Median time to WBC >1.0x10e9/L and neutriphil >0.5x10e9/L recovery was 12 days regardless the filgrastim formulation used (see: Table). However, there was a trend to faster platelet >50x10e9/L recovery and less need for platelet transfusions in the Zarzio group. The use of red blood cell transfusions did not differ for two study cohorts. Patients receiving Zarzio were discharged from the hospital one day earlier compared to those receiving Neupogen (p=0.03). There was a tendency to fewer grade 3 or 4 infectious complications in the Zarzio compared to Neupogen group (16% *vs.* 31%, p=0.09) and significantly less neutropenic fever (7% *vs.* 20%, p=0.03). No severe injection-related complications were observed. No transplantation related mortality was noted.

We conclude that filgrastim biosimilar provides equivalent efficacy in terms of accelerating the neutrophil recovery after autoPBSCT, compared to the original formulation. Differences regarding the duration of hospital stay and the rate of nuetropenic fever require verification in prospective trial.

**P873**

**Biosimilar G-CSF is effective in reducing the duration of neutropenia after autologous bone marrow transplantation**

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Objectives: Granulocyte colony-stimulating factor (G-CSF) is widely used to accelerate haematopoietic recovery after bone marrow transplantation (BMT). Approval of biosimilar G-CSF in Europe has been on the basis of comparable efficacy, safety and quality as the originator product. However, data are not presently available in all clinical settings. This is the first reported use of a biosimilar G-CSF for neutrophil recovery after BMT.

Methods: A total of 23 consecutive patients (12 male, 11 female; mean±SD age 47±13 years) with haematological malignancy (multiple myeloma, n=12; Hodgkin's lymphoma, n=6; non-Hodgkin's lymphoma, n=4; acute myeloid leukaemia, n=1) were recruited at a single-centre. Nineteen patients were receiving their first BMT while it was the second autograft for 4 patients. Mobilisation chemotherapy consisted of high-dose (HD) VEP (n=14), ESHAP (n=4), HD Ara-C (n=3), HD cyclophosphamide (n=2) or ICE (n=1). Patients received biosimilar G-CSF (Zarzio®, Sandoz Biopharmaceuticals) after myeloablative chemotherapy (primarily BEAM or melphalan 140/200 mg/m$^2$ ±bortozemib) with or without radiotherapy followed by autologous BMT. G-CSF therapy was started when absolute neutrophil count (ANC) was <0.5x10$^9$/l and was continued until ANC reached >1.5x10$^9$/l for 3 consecutive days. Response was evaluated after BMT using International Uniform Response Criteria.

Results: Mean±SD number of CD34+ cells collected before transplantation was 10.1±4.0x10$^6$/kg/body weight. After BMT, one patient had a stringent complete response (CR), 7 patients had a CR, one patient a near CR, one patient a very good partial response (PR), 12 patients a PR, and one patient had progressive disease (overall response 96%). Mean

**[P872]**

| | Neupogen (N=35) | Zarzio (N=55) | p |
|---|---|---|---|
| Day of WBC recovery >1.0x10e9/L | 12 (10-15) | 12 (10-13) | 0.19 |
| Day of ANC recovery >0.5x10e9/L | 12 (10-21) | 12 (10-13) | 0.1 |
| Day of PLT recovery >50x10e9/L | 14 (9-22) | 13 (0-19) | 0.05 |
| No. RBC transfusions | 0.77 (0-6) | 0.49 (+/-0.91) | 0.27 |
| No. PLT transfusions | 1.57 (+/-0.81) | 1.39 (+/-0.83) | 0.07 |
| Day of discharge from hospital | 16 (14-25) | 15 (13-30) | 0.03 |
| Infections (grade 3 or 4) | 11 (31%) | 9 (16%) | 0.09 |
| Neutropenic fever | 7 (20%) | 3 (7%) | 0.03 |

recovery to ANC >0.5x10$^9$/l was 13.0±4.0 days. Mean duration until platelet recovery >20 000/ul was 16.1±4.4 days (not achieved in 5 patients at last available assessment). Mean duration of treatment with biosimilar G-CSF was 14.4±5.1 days (range 6-23). Patients required antibiotics on a median of 4 days (range 1-6). Five patients (22%) experienced neutropenic events (neutropenic fever, n=4 and neutropenic enterocolitis and sepsis, n=1). Mean number of days in hospital was 28±6.
Conclusion: Biosimilar G-CSF appears to be effective in reducing the duration of neutropenia in patients undergoing myeloablative therapy followed by autologous BMT.

**P874**
**Collagen receptor-mediated mechanochemical signalling modulates human pro-angiogenic mesenchymal stem/progenitor cells during neovascularisation**
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Despite recent advances in the application of non-hematopoietic stem cells for novel regenerative therapies our understanding of the basic cellular mechanisms involved in therapeutic neovascularization remains incomplete. We have used co-implantation of autologous human mesenchymal stem/progenitor cells (MSPCs) and endothelial colony forming cells (ECFCs) in NSG mice to study formation of patent pericyte-lined and perfused vessels *in vivo*. Matrigel plugs containing either MSPCs, ECFCs, a 20/80 mixture of MSPC and ECFCs, or Matrigel alone, were harvested after 24h to identify potential early *in-vivo* signaling events by antibody microarray analysis. Potential regulatory targets were selected by in-silico interactome analysis and validated *in vitro* at the cellular level.
Expression of the discoidin domain-containing membrane tyrosine kinase receptor 2 (DDR2) in MSPCs was downregulated 2.4 fold in the 20/80 mixture compared to MSPC-only implants. DDR2 has been shown to interact with collagen to sense and modulate matrix stiffness, and to regulate cell migration. Immunofluorescence microscopy revealed components of the mechanotransduction machinery (Paxillin, ILK, p416-Src kinase, cortactin) and pericyte-specific markers (e.g. h1CaP) in MSPCs. Additionally, MSPCs responded to alterations in mechanotransduction elicited by pharmacological manipulation of cytoskeletal integrity with phorbol dibutyrate and the RhoKinase inhibitor Y-27632. We further detected accumulation of DDR2 at the periphery of migrating MSPCs in 2D culture. We also probed MSPC extracts for mRNA and miRNA content in response to different matrix conditions including collagen, Matrigel and human platelet lysate gels. Employing both 2D and 3D culture strategies we identified changes in the levels of DDR2 mRNA and the regulatory miRNAs (29b, 199a, 331) in response to collagen compared to human platelet lysate, together with significant alterations in cell morphology.
Taken together our data point towards a mechanosensitive regulation of MSPC function. Direct or indirect (via miRNA) regulation of the collagen receptor DDR2 could have a central role in modulating MSPC function during stem-cell induced neo-vascularization *in vivo*. In consequence, a collagen-free 3D carrier may be superior in supporting the pericyte-like pro-angiogenic capacity of MSPCs.

**P875**
**Mesenchymal stromal cell for postransplant peripheral cytopenias: feasibility and safety based on compassionate use in 10 patients**
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Introduction: Peripheral cytopenias are a severe complication of allogeneic stem cell transplantation (allo-SCT), often in the context of graft-versus-host disease (GVHD). Standard treatment includes steroids and/or intravenous immunoglobulins (IVIGs), but some cases are resistant. We have recently published the first experience with the use of MSC (Sanchez-Guijo FM *et al.* Transfusion 2011 epub 24 Oct) in four cases. In the current work we report our experience with 10 patients treated by a compassionate use program with third party allogeneic MSC, including two pediatric patients. A standardized infusion protocol was recorded in each intravenous infusion to specifically assess safety and adverse events. Premedication with paracetamol and dexchlorpheniramine was given to each patient prior to MSC administration.
Methods: MSC were expanded from 100 mL of bone marrow from third-party donors (different to that of the allo-SCT) obtained under standard conditions in the Good Manufacturing Practice (GMP) Cell Production Unit at the Hospital Universitario de Salamanca. Quality control of the administered cellular product was performed by immunophenotypic analysis by flow cytometry, *in vitro* differentiation assays to show multilineage differentiation and karyotyping.
Results: Eight adult (mean age: 42.5 years-old, range: 23-61 years) and two pediatric (19 and 34 months-old) patients were treated with MSC for refractory cytopenias after allo-SCT. Reason for MSC therapy were thrombocytopenia (n:5), anemia (n:2), pancytopenia (n:2) and neutropenia (n:1). Total number of doses of MSC administered was 43. Median number of doses per patient was 4 (range: 1-12) and median number of MSC per dose per patient was 1.01x10e6 MSC (range: 0.85-1.45x10e6) in the adult population whereas it was notably higher in the two pediatric patients (4.56x10e6 MSC/dose/patient). No complications nor adverse events recorded regarding cell administration for any of the reported patients in the 43 doses administered were observed. Regarding responses, seven out of ten patients achieved a complete response with full recovery of normal peripheral blood counts. Median follow up is 10 months. One patient did not respond, one patient had a partial response, and the remaining one achieved a complete response and relapsed after 10 months of the last MSC dose.
Conclusion: MSC from a third-party donor is a safe and potentially useful therapeutic approach for patients with refractory cytopenias after allo-SCT.

**P876**
**Mesenchymal stromal cell for the treatment of Behçet's disease: case report**
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Objective: Behçet disease (BD) is a chronic vasculitis of autoimmune origin. A T-lymphocytes helper type-1(Th1) response is documented in affected patients (pts) together with CD56+ NK cells increase. The treatment relies on immunosuppressive therapy and stem cell transplantation (SCT) for patients who fail to respond to conventional therapy. As recurrent vasculitis is the hallmark of the disease, organ damage can occur in severely affected pts, thus precluding SCT. MSC have been successfully used to treat autoimmune disorders. Early *in vivo* results hint at

the ability of MSC to skew a Th1 towards a Th2 response, and to increase CD4+CD25+CD127dim/neg regulatory T cells, thus possibly acting as immunosuppressant.
Methods: After signing written informed consent a 19-year old patient affected with severe, resistant BC received 3rd-party bone marrow-derived MSCs. The patient exhibited BC with ocular, neurological, lung, skin and bowel involvement, and renal impairment precluded her to receive SCT. At the time of MSC infusion she was treated with steroids, azathioprine, methotrexate and cyclosporine. Combined analgesic treatment was needed to control chronic pain. The clinical activity of the disease was assessed with the BD Current Activity Form 2006 (BDCAF). She received 2 i.v. infusions of $10^6$ MSCs/kg body-weight on day 1 and +30 after the first clinical assessment. BDCAF was administered monthly till day +30 after MSC infusion. Immunosuppressive and analgesic treatments were recorded at the same time points. Blood samples were collected weekly to analyze the patient´s lymphocyte and cytokines subsets.
Results: MSCs where infused with no side-effects. No symptomatic benefit was observed after the treatment as documented in the BDCAF. Immunosuppressant and analgesic treatment could not be tapered after MSC infusion. Immunological monitoring of leukocytes subsets did not show any major change into the lymphocyte subpopulations, not even in the T helper/T regulatory ratio, the cytokine pattern showed reduction in IL-8, IL-6 and IL12p70 values whereas IL-17, IL-10, IL-5 and TNF-alpha remained unaltered.
Conclusions: Though clinical feasible and scientifically reasonable, allogeneic MSC infusion in a patient with severe resistant BD did not prove to be of any benefit. Reasons for treatment failure could imply possible MSC rejection or a chronic phase of a long-lasting disease, where acute inflammation plays no longer a major role in the inflammation process.

**P877**

**Infectious complications in patients treated with mesenchymal stromal cells after allogeneic haematopoietic stem cell transplantation**

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Objective: Mesenchymal stromal cells (MSC) are used in clinical trials to treat graft versus host disease (GvHD) after stem cell transplantation (SCT). *In vitro* studies demonstrated MSC broad immunosuppressive activity, documenting interactions with B and T-lymphocytes as well as dendritic cells. As infections represent a major risk after SCT, it is important to comprehend any possible influence of MSC in this context.
Method: We analyzed the clinical course of 11 patients (pts) receiving MSC for GvHD in our Unit between 2009 and 2011. We recorded viral reactivations as measured in whole blood with polymerase chain reaction for 100 days following MSC administration. In pts who had documented viral reactivations in the first 3 days following MSCs infusion the frequency of virus-specific IFN gamma-producing cells was determined through enzyme-linked immunospot assay. Infectious events (viral, bacterial and fungal) requiring cardio-pulmonary support in the 100 days after MSC infusion were also analyzed.
Results: 8 pts presented with viral reactivations after MSC administration, 7 of them while receiving other immunosuppressive treatment. All of them received preemptive treatment according to institutional policy and none developed viral dissemination or overt disease with regard to Epstein Barr Virus, Cytomegalovirus or Adenovirus. Two pts developed viral reactivation the day after MSC infusion. One of them was cytopenic and therefore virus specific IFN gamma-producing cells could not be tested. The 2nd patient showed an increase in CMV-specific T cells after *in vitro* stimulation with viral antigens. One patient experienced a lung infection requiring oxygen administration during the observation time span. The cause of the lung infection was syncitial respiratory virus and the infant recovered in 2 weeks time without further problems.
Conclusion: In our cohort of patients viral reactivation after MSC infusion occurred in 72% of the cases, comparably to other reports dealing with pts affected by resistant GvHD. No patient presented severe form of infection. As all of them were receiving immunosuppression, it is very unlikely to draw firm conclusions about the role of MSC in triggering these events. A single case could be checked for immunological response to viral stimulus and demonstrated virus specific T-cytotoxic lymphocyte activity.

**P878**

**Evaluation of telomerase activity in human bone marrow-derived multipotent mesenchymal stromal cells expanded *in vitro***

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Human *in vitro* expanded multipotent mesenchymal stromal cells (MSCs) have recently gained wide interest in view of their clinical use in several cell therapy approaches. However, *in vitro* cell expansion, which selects for rapidly dividing cells, may increase the risk of spontaneous malignant transformation. Telomerase is a ribonucleoprotein enzyme complex that extends telomere length by adding TTAGGG repeats to the telomeric ends of chromosomes and plays an important role in the control of cell proliferation and tumorigenesis. Telomerase activity is silent in the vast majority of adult human cells; an increase in telomerase activity permits the maintenance or lengthening of telomeres, rendering cells immortal and permitting cancer progression. Even though no critical side effects, including tumour formation, have been described in patients receiving MSCs in clinical trials, the use of MSCs for clinical approaches requires that the bio-safety of these cells be carefully investigated by appropriate and sensitive tests. The aim of this study was to investigate susceptibility to transformation of human bone marrow (BM)-derived MSCs, by measuring the expression of telomerase activity. We analyzed 14 MSC lots at early (P2-P4) and late (P10-P12) passages, all obtained from healthy donors. Results were compared with those obtained in 4 samples of leukaemia blasts from patients at disease diagnosis and in 1 sample of sarcoma cells, as recent evidence suggests that sarcomas arise through aberrant differentiation of MSCs. Telomerase activity was assessed by a quantitative real time PCR-based telomeric repeat amplification protocol (TRAP); results were expressed as attomole of telomerase repeat sequences in 1 µg protein (attomol/µg protein). All MSC samples showed very low levels of telomerase activity both at early- (Mean: $2.6^{-4}$ attomol/µg protein, SD:$\pm 0.87^{-4}$) and late passages (Mean: $0.56^{-4}$ attomol/µg protein, SD:$\pm 3.34^{-4}$). As expected, telomerase activity in leukaemia blasts was higher than in MSC samples (Mean: $0.84^{-1}$ attomol/µg protein, SD:$\pm$ $0.31^{-1}$) while in sarcoma cells the level was intermediate ($0.99^{-2}$). These results suggest that human *in vitro* expanded BM-derived MSCs show very low levels of telomerase activity at early passages and maintain the same levels of telomerase activity also after long term *in vitro* culture, suggesting that these cells do not display susceptibility to malignant transformation.

**P879**

**Elevated oxygen environment boosts MSPC proliferation without senescence aggravation**

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Clinical trials are underway to test the safety and efficacy of mesenchymal stem and progenitor cell (MSPC) in various diseases. MSPC expansion is the prerequisite for dose finding studies as well as for most applications in adult patients. A major safety concern for MSPC expansion is the risk of malignant transformation or premature senescence hampering MSPC function. We have previously shown that long-term expanded MSPCs acquired senescence-related gene expression changes independent of culture conditions (Haematologica 2010). It has been hypothesized that elevated oxygen (20% air O2) contributes to genomic instability and malignant transformation *in vitro*. We therefore analyzed the influence of different oxygen conditions during long-term expansion on MSPC behavior including osteogenic differentiation. A gene panel previously defined as senescence markers was tested for differential expression after varying culture conditions.

Bone marrow-derived MSPCs were expanded in alpha-MEM supplemented with 10% human platelet lysate replacing fetal bovine serum under physiologic conditions (5% $O_2$) or air oxygen (20% $O_2$) until spontaneous cessation of proliferation. Osteogenic induction was analyzed by Alizarin red. RNA was isolated from corresponding early and late passages and analyzed by qRT-PCR for p16ink4a, PARG1, CDKN2B, PTN and MCM6.

In total, MSPCs could be cultured for 5 passages at 30 cells/cm$^2$ and for 10 passages at 3,000 cells/cm$^2$ for up to 85 days resulting in more cumulative population doublings (PDs) of MSPCs at air $O_2$ compared to 5% $O_2$ and in cultures with low compared to standard seeding density. Long-term cultured MSPCs after 40 PDs (air $O_2$) and 35 PDs (5% $O_2$) retained their osteogenic differentiation capacity. Compared to early passages, RT-PCR in late passages revealed an up-regulation of p16ink4a, PARG1 and CDKN2B without specific influence of culture conditions. PTN and MCM6 were significantly down-regulated, mainly in air $O_2$ cultures with high seeding density correlating with diminished cell proliferation compared to low density cultures. There was no evidence of immortalization or malignant transformation.

MSPC large-scale and long-term propagation under animal serum-free conditions at air oxygen in low seeding density was most efficient and safe.

**P880**

**Characteristics of stromal precursor cells studied *in vitro* in patients after allogeneic haematopoietic stem cell transplantation**

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The alterations in stromal precursor cells in patients after allogeneic hematopoietic stem cell transplantation (alloHSCT) are obscure. The aim of the study was to investigate the dynamics of colony forming unit fibroblasts (CFU-F) and proliferative potential of multipotent mesenchymal stromal cells (MMSC) in patients during one year after alloHSCT.

AlloHSCT was performed to 12 patients (6 male and 6 female): 3 patients with CML, 5 with AML, 3 with ALL and 1 with B-CLL. Conditioning was myeloblative in 8 patients and reduced intensity in 4. After informed consent bone marrow was aspirated before conditioning, and 30, 60, 90, 120, 180-240 and 360 days after transplantation. CFU-F was counted in 14 days. MMSC were cultured in alfaMEM media supplemented with 10% fetal calf serum. Cumulative MMSC production was counted after 5 passages.

In 30 days after alloHSCT concentration of CFU-F in bone marrow of patients decreased more than 4 fold (29.6±7.9 per 106 bone marrow cells before alloHSCT and 7.2±3.1–30 days after). Further it gradually increased and one year after alloHSCT mount to 75% (22.4±14.8) of value before transplantation. No differences were revealed between patients received myeloablative and reduced intensity conditioning regimen.

The growth parameters of patients and donors MMSC are significantly different. Even before alloHSCT average time to passage is 1.5 fold longer in patients' MMSC (9.0±0.8 days) than in donors' ones (6.6±0.4). In 30 days after alloHSCT average time to passage increased 3 fold in comparison with donors (20.6±5.0), than it gradually decreased remaining longer than before alloHSCT up to one year. Before transplantation cumulative cell production of MMSC is more than ten times lower than in donors' MMSC. In 30 days after alloHSCT cumulative cell production decreased 1000 fold, and only in one year it attends basic level.

The results demonstrate that stromal precursor cells – MMSC and CFU-F are altered in patients after alloHSCT. MMSC from patients have reduced proliferative potential in comparison with MMSC from donors which does not restored even in one year after alloHSCT.

So the data suggest that stromal cells are violently damaged by alloHSCT procedure and do not regenerate completely even in one year. The status of hematopoietic microenvironment could influence the hematopoiesis in patients after alloHSCT. Further investigations of stromal precursor cells are needed.

**P881**

**Therapeutic neo-vasculogenesis *in vivo* is promoted by oxygen sensing mesenchymal stem/progenitor cells**

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Clinical trials using endothelial progenitor transplantation for therapeutic vasculogenesis have been of rather limited efficiency. We and others have shown that despite hypoxic stimulation, somatic endothelial colony-forming progenitor cells (ECFCs) *in vivo* only form patent vessels in the presence of mesenchymal stem/progenitor cells (MSPCs). Here we show that MSPCs but not ECFCs are the oxygen sensors enabling sustained vasculogenesis after stem/progenitor transplantation *in vivo*.

Adult human ECFCs were isolated from whole venous blood and MSPCs from human bone marrow aspirates. Progenitor phenotype, long-term proliferation, wound repair, migratory and vasculogenic functions were monitored at different oxygen conditions. ECFC and MSPC interaction *in vivo* were studied in immune-deficient NSG mice after subcutaneous co-transplantation using immune histochemistry and TUNEL assays. Chemical and genetic inhibitors of protein synthesis (cycloheximide) and hypoxia-inducible factor-1alpha (HIF-1alpha; YC-1, shRNA) were employed to delineate the role of MSPCs and ECFCs during therapeutic vasculogenesis under hypoxia *in vivo*.

*In vitro* ECFC and MSPC proliferation and function was reduced with declining oxygen levels. ECFCs stabilized HIF-1alpha only at 1% $O_2$, while MSPCs stabilized HIF-1alpha at 1% and 5% $O_2$. In a humanized mouse model, transplanted ECFCs underwent apoptosis after 1 day and attracted mouse leukocyte infiltrates. ECFCs co-transplanted with MSPCs formed perfused human vessels within 7 days independent of matrix protein composition. Perivascular cells, but not ECFCs accumulated HIF-1alpha *in vivo*. Inhibition of MSPC but not ECFC protein synthesis and

HIF-1alpha stabilization prior to co-transplantation completely blocked vessel formation.
Our data show that MSPCs react to the low oxygen environment by HIF-1alpha stabilization and rescue ECFCs from hypoxia-induced apoptosis. Our results demonstrate that MSPCs and not ECFCs act as oxygen sensors during vascular regeneration. This supports a shift of focus from endothelial cells to perivascular cells in regenerative medicine and also as a therapeutic target in anti-angiogenic therapy. Co-transplantation of endothelial and mural precursors appears to be more promising than previous strategies for therapeutic vasculogenesis.

**P882**

**Antileukaemic T-cell response after DC stimulation can be predicted by the composition of T-cell subpopulations: Tcm, Teff as well as by the expression of activation markers and ß integrins on T-cells**

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Problem: Allogenic SCT and DLI are promising T-cell based therapies to cure AML-pts. DC based specific T-cell stimulations could possibly further improve the antileukemic T-cell reactivity -before or after SCT. We could already show, that leukemia-derived DC (DCleu), generated from blasts, are the most effective antileukemic stimulators for T-cells, although not effectful in every case.
Methods: The aim of our study was to further enlighten the role of the composition of T-cell subpopulations Naïve(Tnaive), nonnaïve(Tnon-naïve), effector(Teff), effector memory(Tem) and central memory(Tcm))for the mediation of antileukemic reactions. Moreover we investigated possible shifts of expressions of activation markers (CD69, CD71, CD28, CD137 and CD154) or of beta-Integrins beta 7-Integrin and CD29 on T-cells.
In 0-7 days' mixed lymphocyte cultures (MLC) we stimulated autologous (n=5) or T-cells after SCT(n=6) with leukemia derived DC or blasts and studied their antileukemic reactivity compared to unstimulated T-cells in a functional Fluorolysis assay.
Results: 1. Unstimulated and stimulated T-cells of AML-pts before or after SCT were characterized by higher proportions of Teff (p=0.02) and lower proportions of Tcm (p=0.08) in CD4+ and CD8+ compared to healthy T-cells. Moreover healthy T-cells showed a higher degree of activation (p=0.01-0.002) as well as higher proportions of ß-Integrin positive T-cells (p=0.001-0.05) before stimulation 2. Kinetic studies of AML samples showed that the T-cellular profiles developed during the first 5 days of DC/blast stimulation. 3. Antileukemic functionality of T-cells was achieved in 7 of 11 (64%) cases after DC-stimulation compared to 4 of 10 cases (40%) after blast stimulation. 4. T-cell profiles correlate with antileukemic functionality of DC stimulated T-cells: In general lytically active T-cells present with a higher expression of activation markers (p=0.006-0.05) and higher proportions of Tcm and Teff, ß7+ and CD29+ Tnon-naïve compared to non-lytic T-cells.
Conclusion: Our data confirm the central role of DC in the mediation of antileukemic T-cell response: Cases with lytic activity are characterized by higher proportions of activated T-cells, Tcm and Teff as well as ß7 or CD29 expressing T-cells. Our data contribute to enlighten the role of T cell subtypes in the mediation of antileukemic reactions, to predict their antileukemic function and possibly to identify and separate T cells mediating GvL without GvHD.

**P883**

**Paramunity-inducing Factors have the capacity to improve DC-maturity, proportions of DCleu and early T-cell proliferation, but impair ex-vivo antileukemic functionality and therefore require thorough analyses before application in man**

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Introduction: PINDs consist of attenuated and inactivated viruses of various poxvirus genera (orff (o), avi (a), or both), and are used in veterinary medicine as non-antigen-specific and non-immunising stimulators of the innate immune system against infectious and malignant animal diseases. Their danger signaling interactions could make them promising tools to improve leukemic antigen presentation on DC, generated from AML-patients' blasts and improve the DC-stimulation of antileukemic T-cells.
Methods: The aim of our study was to elicit, whether the addition of different PINDs (Zylexis (ZY, o) Conpind (o, a), HP(a), 3P(o), HP-3P(o, a)) during DC cultures and mixed lymphocyte reactions (MLR) with autologous(n=2), allogeneic (n=3) or T-cells after SCT(n=3) would alter the quality and quantity of DC, composition of T-cell subsets, and/or their antileukemic functionality(AF) as studied by FACS and functional Fluorolysis assays.
Results: 1. Effects of PINDs on DC: a) PINDs generally increased maturity (61 *vs*. 50%, p=.23) and the proportions of DCleu (65 *vs*. 57%, p=.55), but reduced their quantity (30 *vs*. 41%, p=.14) and viability (55 *vs*. 60%, p=.29). b) ZY displayed the best overall performance, particularly benefitting maturity (71 *vs*. 50%, p=.04). 2. Effects of ZY on MLR: ZY induced early T-cell activation (CD69), mainly in the CD4+ subset (+613 *vs*. +170%), while late activation- and proliferation-markers (CD137, CD154, CD28, CD71 and CD25) behaved similar to MLR without ZY. T-cell differentiation to effector-T-cells and regulatory-T-cells was impaired. 3. AF was achieved in 5 of 8 cases after conventional DC-stimulation, compared to only 1 of 8 cases employing ZY (less than in the control with blast-stimulated T-cells (3 of 8)). Additionally, a blast-proliferation of up to 400% was observed under ZY.
Conclusion: Our data show promising results from the usage of PINDs regarding DC-maturity, proportions of DCleu and early T-cell-induction, though resulting in an opposite effect on AF, showing an almost protective capacity towards blasts. This should raise awareness on unspecific influences of PINDs, indicating, that thorough analysis on their whole range of effects in different settings (e.g: in/ex-vivo environment, infectious/malignant) is required, before utilization in man. Nevertheless our results hint the potential of PINDs to evolve into a powerful tool in manipulating immune-processes in the future.

**P884**

**CD4+ as well as CD8+ T-cells addressing leukaemia-associated antigens (LAA: WT1, PRAME, PR1) or minor histocompatibility antigens (HA1) can be identified and mobilised to recognise and kill leukaemic cells expressing from patients with AML**

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Introduction: CD4+ or CD8+ T-cell based immunotherapies address LAA (WT1,PR1,PRAME) or minor histocompatibility

antigens (HA1). We present profiles and functional characteristics of LAA directed CD4+ and CD8+ cells and provide strategies for immunotherapies.
Methods: LAA-peptide specific CD8+ T-cells were identified by MHC-multimer technology (MMT) and spectratyping (n=7). LAA/HA1-protein specific CD4+ T-cells were isolated after stimulation with LAA/HA1-protein loaded CD4-depleted MNCs as APC (n=6). Cells were characterized by PCR, FACS, (intracellular) cytokine, Fluorolysis/Chromium release assays. Results: 1.CD8+ T-cells: a.LAA-peptide specific CD8+ T-cells were found in all of 7 AML-pts after SCT. In 5 of 7 cases two different types of LAA-specific CD8+ T-cells were simultaneously detected. b.4 of 5 pts with two different types of LAA-specific CD8+ T-cells showed long lasting remissions. c.MMT selected LAA-specific CD8+ T-cells revealed a restricted TCR Vß-repertoire. 2.CD4+ T-cells: a.We provide a LAA/HA1 stimulation system using LAA/HA1-protein-loaded CD4 depleted MNCs as APC. b.We provide a refined cloning system for proliferating, CD40L+CD4+ T-cells after LAA/HA1 stimulation. c.We show that in general CD4+ T-cells can mediate antileukemic activity and differentially compare the grade of cytotoxic activity in different CD4 subsets. d.Cellular profiles of stimulated/cloned as well as CD4+ T-cells isolated directly from a patient in general revealed an effector/effector-memory profile with a predominant inverse correlation of antileukemic activity with proportions of regulatory T-cells. e.Specific cytokine release in T-cells was seen not until at least 7 LAA/HA1 stimulation passages.
Conclusion: We identified LAA/minor antigen specific CD4+ and CD8+ T-cells in AML pts, demonstrate their anti-leukemic function and provide stimulation and cloning strategies, that in general qualify as immunotherapeutic tools and enable a monitoring of antigen specific T-cells. We show, that a high antileukemic functionality or stable remissions correlate with high proportions of LAA-specific CD8+ and CD4+ T-cells and that certain enriched CD4+ subtypes might be even more promising candidates for GvL- without GvH-reactions. Future strategies could focus on an enrichment of anti-leukemia directed T-cells before or after stimulation with LAA/HA antigens or the depletion of certain unwanted, inhibitory T-cell fractions.

**P885**

**Clinical relevance of mRNA overexpressions of the leukaemia-associated antigens WT1, PRAME and PR1 in patients with acute myeloid leukaemia: an analysis of (co)expressions in different cellular compartments, subtypes or stages**

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Introduction: LAA could qualify as targets for immunotherapies.
Methods: We studied the overexpression of WT1, PRAME, and PR1 in 88 AML pts in different stages/prognostic subgroups and cellular compartments (PB, BM) of AML by real time PCR.
Results: 1. RNA-overexpression was found in 81% of AML-pts for WT1, in 87% for PRAME and in 55% for PR1. 2. Parallel/simultaneous expression of 2 LAAs was found in 70%, of 3 LAA in 43% of single pts at first diagnosis (dgn). 3. Highest LAA-overexpression rates were found at dgn, followed by persistent disease(pers)/relapse and CR. Adjusting RNA expressions to morphological blast counts revealed highest expression rates for all LAA in pers and in addition hints for persisting LAA expressing blasts in 4 of 6 cases in CR. 4. Higher relative overexpression rates of all 3 LAAs were found in PB compared to BM ($p \leq .001$). Parallel analyses of pts' PB and BM-samples showed (significantly) higher overexpression rates of PR1 ($p<.02$), WT1 ($p<.34$) and PRAME ($p<.63$) in PB compared to BM. 5. Prognostically more 'favourable' cytogenetic risk or pAML appearances could be correlated with a (significantly) lower expression of PRAME ($p<.02$) and WT1 ($p<.27$) and higher expression of PR1 ($p<.16$). 6. Cut-off analyses revealed that 100/70% of pts with a more than 400/2500-fold PRAME overexpression in BM/PB-samples, but only 15/13% of pts with a less than 400/2500-fold overexpression and for PB with PR1 pts with more than 100/80-fold overexpression presented with an in general more favourable pAML appearance. Pts treated successfully by FLAMSA immunotherapy were characterized by higher PR1 and lower PRAME RNA-overexpression compared to pts with no response.
Conclusion: WT1, PRAME and PR1 are regularly overexpressed in AML pts at first dgn: At least one of these three LAA is overexpressed in every given AML case. 70% of cases overexpress simultaneously 2 LAAs, pointing to single blasts with multiple or different blasts with single LAA overexpressions. High PRAME and low PR1 expression rates correlate with unfavourable prognosis. Higher overexpression rates of all three LAAs are found in PB compared to BM. We recommend to analyse PB as well as BM with our refined (blast-adjusted) PCR-technology using three different LAA to detect and monitor clonal or oligoclonal AML in the course of the disease and to quantify the tumor load in different compartments. Our findings contribute to develop refined LAA-directed immunotherapies to treat AML-patients.

**P886**

**Successful generation of p190-BCR ABL-specific T-cell lines for prophylaxis/treatment of minimal residual disease in HSCT recipients with Ph+ acute lymphoblastic leukaemia**

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Recent studies by our groups have demonstrated the presence of BM-homing, BCR-ABL specific cytotoxic T cells (CTL) in Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL) patients during sole Imatinib mesylate maintenance treatment, that inversely correlated with minimal residual disease (MRD). This observation supports the notion that anti-tumor T lymphocytes may effectively participate in the control of Ph+ leukemic proliferation, and represents the rationale for a BCR-ABL-targeted cell therapy approach to prophylactically treat leukemic relapse after HSCT in patients with Ph+ALL. The aim of this study was to evaluate the feasibility of expanding BCR-ABL specific CTL from HSCT donors, to be employed as specific DLI after HSCT for Ph+ALL. We conducted scale-up experiments to validate an *in vitro* culture method to expand BCR-ABL specific CTL from HSCT donors, by peripheral blood mononuclear cell (PBMC) stimulation with 9-20mer peptide pools derived from the p190 BCR-ABL fusion region. T-cell lines, that included a median 70% CD4+ and 29% CD8+ T lymphocytes, were successfully generated from 5/6 individuals. The T-cell lines showed specific INFg production in Elispot assays consistently higher (median 130 SFU/10e5 cells, range 0-198) than non-cultured donor PBMC (median 4 SFU/10e5 cells). In a standard 51chromium release assay, 5 of 6 T-cell lines presented specific cytotoxic activity against PHA blasts pulsed with BCR-ABL peptide mix (median lysis 30%, range 6-65), CD3-redirected activity against P815 cells (median lysis 38%, range 36-52) with minimal residual alloreactivity (median lysis 4%, range 0-15). Our data indicate that BCR-ABL-specific T-cell lines with limited alloreactivity may be expanded from HSCT donors after stimulation with BCR-ABL fusion region-derived peptides. Their efficacy in containment of MRD after allogeneic HSCT for Ph+ALL remains to be evaluated in clinical trials.

**P887**

**Evaluation of the receptor for hyaluronic acid mediated motility as a tumour-specific antigen in AML**

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Therapies aimed at eliminating the leukemic stem cell (LSC) could be used to cure Acute Myeloid Leukemia (AML). As chemotherapy often fails to kill the quiescent LSC, the potent Graft-versus-Leukemia effect, as observed in hematopoietic stem cell (HSC) transplantation, supports a role for immunotherapy in the treatment of AML, using cytotoxic T cells recognizing an AML specific antigen on the LSC. It was shown in vaccination trials that RHAMM (Receptor for Hyaluronic Acid Mediated Motility) generates a cellular immune response in patients with AML. However, it is not clear whether this response actually targets the true LSC. Therefore, we evaluated the expression pattern of RHAMM in LSCs compared to HSCs of healthy donors, the latter population prior to and after *in vitro* and *in vivo* expansion.

We isolated two subpopulations from bone marrow/apheresis of both AML patients (diagnosis or relapse) and healthy donors by Fluorescence-Activated Cell Sorting: CD34+CD38- (LSCs) and CD34+CD38+ from AML patients, and their normal counterparts from healthy donors. Real-time qPCR could not demonstrate significant expression of RHAMM in HSCs of healthy donors. Overexpression could be clearly visualized in the AML samples, but strikingly, the overexpression in the LSC was minimal, compared with the CD34+CD38+ fraction of the same patients.

Subsequently, human cord blood-derived CD34+ cells were isolated and expression of RHAMM was compared in fresh, non-expanded cells versus cells cultured for 7 days on the OP9 stromal cell line: a clear up-regulation of RHAMM was observed in the expanded CD34+ HSCs compared with baseline expression. To evaluate RHAMM expression during *in vivo* engraftment, we are currently assessing CD34+ cells from cord blood in a NOD scid gamma (NSG) mouse model.

In conclusion, we were able to confirm that healthy resting HSCs do not express RHAMM. However, our data suggest that immunotherapy targeting RHAMM will not recognize the true LSC and might be capable of eliminating only the progeny of the LSC. Strikingly, *in vitro* expansion of healthy HSCs causes a robust up-regulation of RHAMM. This might be a major limitation for immunotherapy targeting RHAMM in the clinical setting of a stem cell transplantation, because RHAMM-specific cytotoxic T cells might not be able to discriminate engrafting donor HSCs versus LSCs. Therefore, RHAMM may not be an ideal AML specific antigen and we advocate caution to use RHAMM-directed immunotherapy in patients.

**P888**

**Dual transgenesis of T-cells with a CD44v6 CAR and a suicide gene for the safe eradication of myeloid leukaemia and myeloma**

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Introduction: The recent and extraordinaire clinical successes of T cells redirected with chimeric antigen receptors (CARs) indicate this strategy as the new frontier for the immunotherapy of hematological malignancies. A widespread application of the strategy is however limited by the current lack of CARs for diseases different from B-cell tumors. The variant isoform 6 of CD44 (CD44v6) is expressed on acute myeloid leukemia (AML) and multiple myeloma (MM) cells, but not on hematopoietic stem cells (HSC). The possible involvement of CD44v6 in chemoresistance and relapse makes it an attractive target for disease eradication.

Aim: To develop a CAR strategy for safely eradicating AML and MM through the elimination of chemoresistant cells.

Results: We recently found that CD44v6 is crucially involved in stroma-induced chemoresistance and *in vivo* tumorigenicity in both AML and MM. We therefore constructed a novel CAR specific for CD44v6 including the CD28 endodomain (CDD4v6 CAR). To provide a safety switch in case of toxicity, the CD44v6 CAR was cloned in a LV carrying a bi-directional promoter for its co-expression with the HSV-tk suicide gene. After LV transduction, T cells were highly cytotoxic against autologous primary AML and MM cells, and could be ablated with the prodrug ganciclovir. In the presence of chemoresistance-inducing stroma, CD44v6-redirected T cells completely cleared tumor cells. Once infused in NSG mice, redirected T cells persisted long term and eradicated AML (THP1 cells and autologous primary cells) and MM xenografts (MM1.S cells). Interestingly, the eradicating effect was dependent on both the CD28 endodomain and on CD28 costimulation used for transduction (beads). As expected, CD44v6-redirected T cells were not cytotoxic against HSC, however they recognized mature monocytes, suggesting the need of a suicide gene for controlling late toxicities. Since the rapidity of suicide-gene activation is critical to ensure the safety of potent effectors such as CAR-redirected T cells, we explored the novel inducible form of caspase 9 (iCasp9) as an alternative to HSV-tk and crucially demonstrated a much faster kinetics (hrs *vs* days).

Conclusions: CD44v6-redirected T cells have the potential to selectively eliminate AML and MM cells that resist chemotherapy. Once the eradicating effect is achieved, CD44v6-redirected could be ablated through suicide-gene activation not to interfere with full hematopoietic reconstitution.

**P889**

**Investigation of *in vitro* experimental models for immunogenicity assessment of the of cord blood-derived human multipotent mesenchymal stromal cells**

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In virtue of their biological properties human multipotent mesenchymal stromal cells (MSC) have been identified as a promising therapeutic tool in a variety of human diseases. Results reported in seminal studies prompted the hypothesis that MSC are immune privileged as they do not activate immune cells capable of rejecting them. However, subsequent reports have demonstrated that activated NK lymphocytes mediate cytolysis of MSC even though MSC suppress functions of resting NK cells. Moreover, an experimental model of HSCT documented that donor-derived murine MSC are immunogenic in an allogeneic host and stimulate donor graft rejection. We employed an *in vitro* approach to investigate the interaction pattern of cord blood-derived MSC with immune cells obtained from PBMC of a potential allogeneic host. Several parameters of the cell-mediated immune response were evaluated in effector cells derived from PBMC/MSC mixed cultures in comparison with PBMC cultured in complete medium alone (ctrl-cultures, spontaneous activation).

Results documented a remarkable variability in respect to MSC immunogenicity and immunomodulatory properties. MSC stimulated the proliferation of CD4 T-lymphocytes in 9 of 10 exp ($p<0.05$) including both conventionally activated CD25 and CD25/foxp3 Treg, and CD8 T-cells in 7 of 10 exp, while they

inhibited the NK proliferation in 7 of 10 exp (p<0.025). In 4 of 10 exp, effectors from mixed cultures displayed increased MSC-directed cytotoxic activity compared with ctrl-cultures (p<0.025), while in 4 of 10 exp we observed an inhibitory effect (p<0.05). MSC variably induced potential cytotoxic activity (CD107a expression) in a small % of T-CD4, T-CD8, T-CD56 and NK cells.
Results of mixed cultures performed at least in duplicate employing the same lot of MSC with PBMC of the same donor obtained at different time points, also showed intra-experimental variability. The presence of neutralizing anti-HLA-G mAb (G1/G5 specific) in mixed cultures strongly increased MSC-directed cytolysis in one exp and was irrelevant in another exp. MSC suppressed NK activity in 2 of 4 exp and enhanced it in 1 exp; a similar pattern was observed investigating CD3-redirected CTL activity. Altogether, results suggest that MSC may interact with T and NK lymphocytes with an inhibitory or activating pattern depending on the immune cells state of activation. Activated T or NK cells able to mediate cytolysis of MSC could counteract their immunomodulatory properties.

**P890**
**CTLA-4 polymorphisms and clinical outcomes from HLA-matched sibling allogeneic haematopoietic stem cell transplantation**
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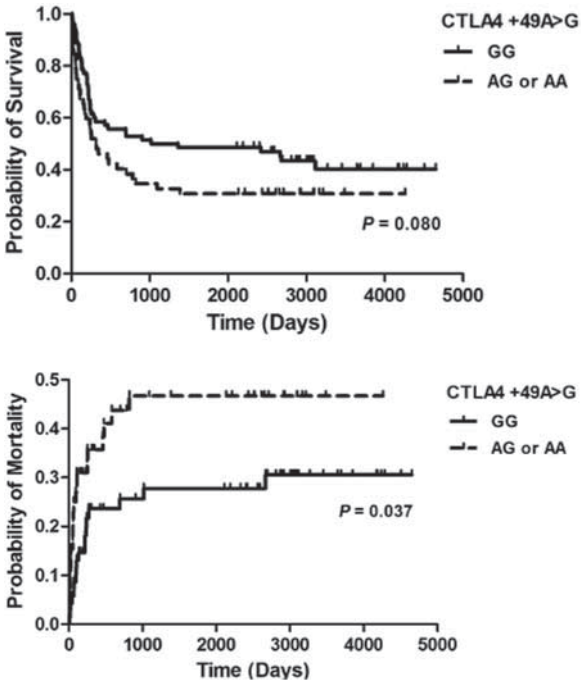
Objectives: CTLA-4 is known as a negative regulator of T cell activation. To evaluate whether the CTLA-4 polymorphisms are related to outcomes of allogeneic hematopoietic stem cell transplantation, we analyzed an association between these polymorphisms and clinical outcomes in patients treated withHLA-matched sibling stem cell transplantation.
Methods: Patients (N =122) with a hematologic malignancy or aplastic anemia who underwent allogeneic stem celltransplantation from sibling donors at Seoul National University Hospital between July 1998 and June 2005 were included. The-318C>T and +49A>G polymorphisms of CTLA-4 gene were genotypedusing peripheral blood DNA of 122 patients andtheirdonors.
Results: Two donors were excluded because of a failure of genotyping, and a total of 242 subjects were genotyped. The genotypes of +49A>G in 122 recipients were AA in 6, AG in 46, and GG in 70; the genotypes in 120 donors were AA in 8, AG in 45, and GG in 67. The frequencies of the A and G alleles in all 242 subjects were 25% and 75%, respectively. In addition, the genotypes of -318C>T in 122 recipients were CC in 107, CT in 14, and TT in 1; the genotypes in 120 donors were CC in 95, CT in 24, and TT in 1. The frequencies of the C and T alleles in all 242 subjects were 91% and 9%, respectively. The presence of A allele in recipient +49A>G showed a trend for worse overall survival (OS) (1015 days for GG *vs.* 313 days for AG or AA; P=0.080), and significantly increased treatment-related mortality (TRM) when compared with the presence of G allele only (P=0.037). However, donor +49A>G polymorphism had no association with OS and TRM. Similarly, both recipient and donor -318C>T polymorphism had no influence on these clinical outcomes. Early mortality rate within 180 days after transplantation was significantly higher in the presence of at least one A allele in recipient +49A>G polymorphism (P=0.048). In addition, there were significantly more infection episodes including neutropenic fever and microbiologically documented bacteremia in recipient +49AG or +49GG alleles during neutropenic phase after allogeneic stem cell transplantation (P=0.002).




Conclusions: In conclusion, the recipient +49A>G polymorphism in CTLA-4 gene is related to TRM after allogeneic stem cell transplantation. This finding needs further validation in well-designed prospective clinical trials.

**P891**
**Monitoring of IL2R, TNFR1, CCL8, HGF, and IL8 serum concentrations and their role on GvHD occurrence and HSCT outcome: a retrospective single-centre analysis**
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Objectives: The aim of this study was to monitor several cytokines early after HSCT in T-cell replete, T-cell deplete and autologous HSCT and to establish their specific roles on GVHD occurrence and TRM.
Patients and Methods: We analysed the serum levels of IL2R, TNFR1, CCL8, HGF, IL12, and IL8 before and after HSCT in 170 paediatric patients of allogeneic T-cell replete grafts, 13 patients of haploidentical T-cell depleted grafts and 14 patients underwent autologous HSCT. Serum cytokines were analysed on day -1, days +1, +7, +14, +21, +28 and +60 by a human 12-Bio-Plex assay kit in triplicate experiments (Bio-Rad Laboratories, Milan, Italy). Student's T test was used for group comparisons of continuous variables. Thereafter, acute, chronic GVHD and 5-y TRM were calculated according to cumulative incidence (CI) probabilities.
Results: The analysis of IL2R and TNFR1 serum levels did not differ among recipients of T-replete or deplete allogeneic grafts, while they were higher for allogeneic graft recipients compared to autologous grafts at each time point (P<0.000). The IL8 serum level was significantly higher on days +1, +7 and +14 for allogeneic transplant *vs.* autologous transplants. IL12p70 was significantly higher on day +1 for T replete grafts compared to T-cell depleted grafts (P=0.01), while no differences where observed between allogeneic and autologous grafts at any time points. The CCL8 serum concentration was significantly higher on day +28 for allogeneic transplant recipients compared to autologous recipients (P=0.02). HGF serum levels were

significantly higher for allogeneic *vs.* autologous transplants at days +1, +7, +14, +21 and +28. To evaluate GVHD incidence, we normalized the serum cytokine increase according to the baseline value for each patient receiving unmanipulated transplants. The crude aGVHD incidence (grade III-IV) was significantly higher for patients with higher IL2R at days +7 and +14, higher CCL8 at day +14, and higher levels of TNFR1 at days +14, +21 and +28. The CIs of aGVHD grade II-IV, cGVHD and TRM were 57.7% *vs.* 28%, 23.8% *vs.* 16% and 15.8% *vs.* 4% (all with P<0.001), for patients having CCL8, IL2R and TNFR1 over the median fold increase, respectively.
Conclusion: Our data shows a different pattern of cytokine storm in myeloablative autologous and allogeneic transplants and that an early increase of pro-inflammatory cytokines is related to severe aGVHD occurrence, translating later into higher TRM risk.

**P892**
**T-cells specific for human c-MYC exhibit anti-lymphoma activity in mice**
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The proto-oncogene c-myc is overexpressed in a variety of cancers. In Burkitt´s lymphoma it becomes constitutively activated by chromosomal translocation. Targeting the driving oncogenic machinery of a malignancy by antigen specific T-cells is an attractive strategy for several reasons: (i) The oncogene is expressed within all tumor cells. (ii) in case of c-myc, silencing of oncogenic activity results in growth arrest, differentiation or cell death and therefore (iii) due to oncogene addiction of malignant cells, development of antigen loss variants is less likely. The homology between murine and human c-MYC is approximately 87%. Several non-homologous regions of the human c-MYC protein give rise to immunogenic epitopes in H2b context according to peptide prediction. Mice immunized with peptides derived from non-homologous regions of the human c-MYC protein developed a c-MYC specific T-cell response. As demonstrated by intracellular IFN-gamma staining this response comprised CD4+ and CD8+ T-cells and frequency ranged from 0.5-3.0% of CD3+ T-cells. Animals immunized with a H2Db restricted c-MYC peptide specifically lysed peptide loaded target cells *in vivo*. Transfer of 0.1 Mio human c-MYC overexpressing lymphoma cells in mice immunized with a c-MYC specific CD8 epitope resulted in 30% rejection of the lymphoma. In contrast, immunization of recipient mice with irrelevant or with CD4 restricted epitopes failed to rescue mice from lymphoma challenge. Depletion of T-regulatory cells by antibody treatment prior to immunization increased survival rate up to 60% after lymphoma challenge. Frequency of c-MYC-multimer specific CD8+ T-cells after immunization was approximately 0,2-2% of the CD8+ compartment. After lymphoma challenge animals pertained or expanded the number of c-MYC specific CD8+ cells. However, adoptive transfer of CD90.2 selected T-cells after prime-boost immunization of GFP-transgenic donor mice into lambda-hu-c-myc mice resulted in long term persistence of GFP positive cells in the peripheral blood for up to 1 year, but was not associated with improved survival compared to mice receiving T-cells from irrelevant peptide immunized donors. We conclude that c-myc specific T-cells undergo peripheral tolerization due to the permanent expression of c-myc within the B-cell compartment. Protection from lymphoma challenge in lymphoma transfer experiments therefore may be a result of MYC acting as foreign antigen in this setting.

**P893**
**Plasma exosomal RNAs as noninvasive biomarker of marrow engraftment after allogeneic haematopoietic stem cell transplantation**
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Introduction: Recent studies have demonstrated that a variety of cells releases exosomes, which include mRNAs and microRNAs (miRNAs). Although naked RNAs are unstable, exosome-encapsulated RNAs (eRNA) are stable even in serum or plasma. Moreover, the levels and composition of plasma eRNAs may correspond to certain physiological or disease conditions by representing tissues origins of exosome. We assumed that bone marrow precursor cells also produce exosomes, which release to peripheral blood. Thus, by measuring bone marrow precursor cells-specific mRNAs in plasma exosome, bone marrow condition may be evaluated without invasive bone marrow aspiration. This study has focused on uncovering the potential role of extracellular eRNAs after allogeneic hematopoietic stem cell transplantation (allo-HSCT) as non-invasive biomarkers for bone marrow engraftment.
Patients and Methods: Plasma was collected form 18 patients before, 14, and 28 days after allo-HSCT. Using 300 µL of plasma, exosome was isolated and poly(A)+ mRNA was purified. Then quantitative PCR was carried out to amplify control (ACTB and B2M), myeloid (DEFA3 and SRGN), erythroid (HBB and UROD), and megakaryocyte (CD61 and ITGA2B) -lineage specific mRNAs. The results were compared with complete blood count (CBC).
Results: Among 2 sets of genes, DEFA3 and HBB were better than SRGN and UROD, respectively. The performance of CD61 was similar to that of ITGA2B. The slope of mRNA increase (DeltaCt/day) was well correlated with the slope of WBC (DeltaWBC/day) with r2 more than 0.9 for both DEFA3 and SRGN. The DeltaCt/day of HBB was correlated with the slope of reticulocyte (DeltaRet/day) with r2 0.47. The DeltaCt/day of CD61 and ITGA2B were correlated with the slope of platelet (DeltaPlt/day) with r2 more than 0.39. More interestingly, the increase of mRNAs happened much earlier than that of CBC.
Conclusion: Our results indicate that plasma eRNAs analysis could be a useful tool for the evaluation of bone marrow recovery and might be alternative to conventional bone marrow aspiration which forced mental and physical burden.

**P894**
**Impaired inhibition on B-cell activation by bone marrow-derived mesenchymal stem cells with decreased CCL2 expression in MRL/lpr mice**
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Objective: Bone marrow-derived mesenchymal stem cells (BM-MSCs) are multipotent cells characterized by immunomodulatory properties and are therefore considered a promising cell therapy for autoimmune diseases. It has been shown that BM-MSCs from healthy donors are able to inhibit the activation of B cells *in vitro* and *in vivo*. Our previous study revealed that BM-MSCs from patients with systemic lupus erythematosus (SLE) possessed some dysfunctions. This study aimed to assess whether inhibition on B cell activation by BM-MSCs in MRL/lpr mice, an animal model of SLE, was impaired and the possible mechanisms involved in this process.
Methods: BM-MSCs were isolated and expanded from either C57BL/6J or MRL/lpr mice. The effects of BM-MSCs on the proliferation and differentiation to plasma cells of normal splenic B cell isolated from C57BL/6J mice were evaluated *in vitro*. And the differential expression of CCL2 on BM-MSCs from C57BL/6J or MRL/lpr mice was detected. Lupus mice were

treated with these two different BM-MSCs respectively, and the levels of serum autoantibodies and immunoglobulin deposition in the kidney were monitored.
Results: BM-MSCs from C57BL/6J mice inhibited the proliferation and differentiation to plasma cells of B cells *in vitro*. This inhibitory effect was mediated by soluble factors, including CCL2, as neutralizing CCL2 could abolish the suppressive effect on B cells mediated by normal BM-MSCs. Inhibition on B-cell proliferation and differentiation by BM-MSCs from MRL/lpr mice was impaired, partially resulted from down-regulated expression of CCL2. The addition of processed CCL2 restored inhibitory effects of BM-MSCs from MRL/lpr mice on B-cells. *In vivo* treatment with BM-MSCs from MRL/lpr mice did not reduce the levels of serum pathological antibody production and immunoglobulin deposition in the kidney compared with treatment with BM-MSCs from C57BL/6J mice.
Conclusions: Our findings suggest that inhibitory effects of BM-MSCs on B-cell are mediated by soluble factors including CCL2. Impaired inhibition of BM-MSCs from MRL/lpr mice on B-cell maybe attributes to the down-regulation of CCL2 expression, which may play an important role in the pathogenesis of SLE.

**P895**
**Multipotent Vdelta2-negative gamma-delta-T-cells after CMV-reactivation in allogeneic stem cell transplantation**
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Human cytomegalovirus (CMV) infections and relapse of disease remain major problems after allogeneic stem cell transplantation (allo-SCT), and adoptive transfer of antigen-specific alpha-beta-T-cells has been proposed as a treatment strategy. However, transfer of antigen-specific alpha-beta-T-cells is substantially hampered by their MHC-restriction and the fact that sufficient numbers of e.g. CMV-reactive alpha-beta-T-cells can be exclusively isolated from CMV-positive donors. Great promise as an alternative strategy, with a potential use for very broad patient population, arises from the non-MHC-restricted target-recognition of cancer cells and virally-infected cells by gamma-delta-T-cells. Therefore, we investigated the role of gamma-delta-T-cells after allo-SCT and CMV infection.
An increase in selectively Vdelta1-positive gamma-delta-T-cells was observed in CMV-reactivating patients from CMV-positive conventional donors. Even more important, gamma-delta-T-cell expansions were also observed after CMV-negative cordblood transplantation. However, in contrast to expansions from CMV-positive donors, gamma-delta-T-cells from cordblood donors did not only include Vdelta1-positive but also Vdelta3-positive cells. Expanded Vdelta2-negative gamma-delta-T-cells were not only able to lyse CMV-infected fibroblasts, but also to partially mature dendritic cells (DCs) and to kill primary leukemic blasts.
In order to investigate whether these different functions observed in gamma-delta-T-cells which expand after CMV-infection are restricted to one cell type or to a diverse gamma-delta-T-cell repertoire which expands after CMV-infection, gamma-delta-T-cells were further cloned by limiting dilution. CMV- and leukemia-reactivity were restricted to the same clonal population and separated from DC-maturation capacities of Vdelta2-negative T-cells. Finally, gamma-delta-T-cell receptor (TCR)-gene transfer experiments indicated that here-isolated Vdelta2-negative gamma-delta-TCRs selectively mediate anti-leukemia-reactivity and that activation of T-cells depends also on the presence of NKG2D. This makes CMV-reactive gamma-delta-T-cells e.g. from cordblood donors as well as individual Vdelta2-negative gamma-delta-TCRs interesting candidates for adoptive immunotherapy strategies.

**P896**
**HLA-A*2402-restricted and WT1 235-243-specific T-cell receptor gene transduced CD4+ T-cells diversely enhance the anti-leukaemia functionality mediated by similarly redirected CD8+ T-cells using the identical TCR gene transfer**
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Purpose: Longevity of infused cancer-specific T cells has been underscored for the better clinical outcome of redirected T-cell-based anticancer adoptive immunotherapy using cancer antigen-specific T-cell receptor (TCR ) gene transfer. To this end, in this study, we focused on helper functions mediated by similarly redirected CD4+ T cells using the identical TCR gene transfer.
Methods: Using our novel TCR gene transfer vector encoding silencers for endogenous TCRs (WT1-siTCR vector), we retrovirally transduced HLA-A*2402-restricted and WT1 235-243-specific TCR alpha/beta genes into CD4+ T cells. Then we in detail examined helper functions mediated by those redirected CD4+ T cells; (i) cognate epitope-responsive cytokine production, (ii) impacts on the epitope-specific cytotoxicity, epitope-responsive proliferation and formation of CD45RA+CD62L- central memory T-cell (Tcm) subset mediated by WT1-siTCR transduced autologous CD8+ T cells, (iii) epitope-responsive chemokine production, (iv) anti-leukemia reactivity mediated by similarly redirected CD8+ T cells in the presence or absence of those redirected CD4+ T cells.
Results: In response to cognate WT1 epitope, WT1-siTCR-transduced CD4+ T cells produced Th1 cytokines such as IL-2, IFN-gamma and TNF-alpha, but not IL-10 or IL-17 in a HLA-A*2402 restricted fashion. In our transfection system, those redirected CD4+ T cells hardly displayed intracellular FoxP3, a key signature of regulatory T cell. In the presence of these helper functions mediated by redirected CD4+ T cells, WT1-siTCR-transduced CD8+ T cells successfully enhanced the reactivity both to cognate epitope and leukemia cells, including cytocidal activity, IFN-gamma production, proliferation and formation of Tcm subset. Redirected CD4+ T cells produced epitope-responsive CCL3 and CCL4, resulted in migration activity mediated by similarly redirected CD8+ T cells. Finally, WT1-siTCR transduced CD8+ T cells implemented the enhanced cytocidal activity, proliferation and formation of Tcm subset against autologous leukemia cells, but not normal cells, only in the presence of redirected CD4+ T cells using the identical WT1-siTCR vector.
Conclusion: Redirected CD4+ T cells seem of multifactorial help for similarly redirected CD8+ T cells using the identical TCR gene transfer. Notably, the augmented Tcm subset formation might be able to contribute to the persistent anti-leukemia efficacy *in vivo*. Thus, further studies are underway.

**P897**
**Rejection of high-grade B-cell lymphoma in mice by T-cells specific for CD19**
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Targeting self antigens by specific T cells for anti-tumor therapy bears the risk of fatal autoimmunity. Appropriate self antigens are characterized by restricted expression in a specific cell compartment and are ideally highly expressed in malignant cells. Potential autoimmunity against those antigens must be tolerable for the host. The CD19 molecule is a transmembrane

protein whose expression is restricted to B cells in mouse and human. CD19 is expressed on virtually all B-cell malignancies and is known to contribute to delivering a survival signal to B cells. Targeting CD19 by antibodies appears to be effective in disease control making it an interesting target for T-cell therapy.
Using homozygous CD19cre mice as a model for CD19 deficiency we show that transfer of lethal doses of lambda-myc transgenic B-cell lymphoma cells (0.1-1.0x$10^6$ 291cells), which form lymphomas in 85% of wild type recipients, can be rejected long term (100 days) in up to 60% (15/25) of CD19 deficient mice. After s.c. injection 8/25 of CD19-/- animals transiently form tumors at the site of injection which eventually disappear as a sign of rejection. Depletion of T cells prevents rejection of lymphoma cells in all CD19-deficient animals (16/16). In case of shrinking tumors as well as in some outgrowing tumors we observed an infiltration by CD4 T cells, which was completely absent in wild type recipients.
Heterotypic prime-boost-vaccination of CD19-deficient mice using a CD19 expression vector and long peptides results in a strong CD4 T-cell response, which improves rejection of 291 lymphoma cells. A CD19-specific CD4 T-cell line (T27), which detects CD19+ lymphoma cells *in vitro*, as indicated by IFN-gamma ELISPOT and ELISA assay, could be generated from vaccinated animals. In a prophylactic setting intravenous application of 1x$10^6$ T27 cells prior to transfer of 1x$10^6$ 291 cells enhanced the survival of CD19-deficient mice.
Taken together our data indicate that CD19 serves as a T-cell rejection antigen in our model and provide evidence that CD4 T cells are important for rejection of lymphoma cells.

**P898**
**Antileukaemic T-cell responses can be predicted by compositions of regulatory T-cell subpopulations, especially with respect to regulatory effector-memory and regulatory CD8+ T-cells**
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Problem: Allogenic SCT and DLI are promising T-cell based therapies to cure AML-pts. Dendritic cells (DC) based specific T-cell stimulations could possibly further improve the antileukemic T-cell reactivity before or after SCT. We could already show, that leukemia-derived DC (DCleu), are the most effective antileukemic stimulators for T-cells, although not effectful in every case.
Aim: The aim of our study was to further enlighten the development and composition of regulatory T-cell (Treg) subpopulations especially in the context of T-cells' antileukemic functionality: Naïve (Tnaive reg), central memory (Tcm reg), effector (memory) Treg (Teff/emm reg), regulatory ectoenzymes (CD39) or DC contact relevant Tregs (CD152) for the mediation of antileukemic reactions were analysed. In mixed lymphocyte cultures (MLC) we stimulated autologous (n=6) or allogeneic T-cells (n=1) or T-cells after SCT (n=5) with DCleu or blasts and studied their antileukemic reactivity compared to unstimulated T-cells in a functional Fluorolysis assay.
Results: 1. Unstimulated T-cells of AML-pts before or after SCT were characterized by significantly higher proportions of Treg subpopulations (CD4+Treg, CD8+Treg, Tnaive reg, Teff/em reg, Tcm reg, CD39+ Treg) compared to healthy T-cells. 2. 'DC' (as well as blast) stimulated T-cells of AML-pts showed significantly higher proportions of Treg subpopulations (Teff/em reg Tcm reg Tnaive reg CD8+ Treg CD4+ Treg) and similar expression profiles of the other surface markers compared to healthy probands. 3. Kinetic analyses over time revealed a continuous increase of all Treg subpopulations during DC and blast stimulation in AML as well as healthy T-cells. 4. Functional correlations revealed significantly higher proportions of CD8+ Treg, Teff/em reg and tendentially CD39+ T-cells in cases without antileukemic activity after DC-, but not after blast stimulation. 5. Low proportions of Treg subpopulation correlate with antileukemic functionality of DC stimulated T-cells: Cases with <60% of CD8+ Treg, <60% of Teff/em reg and <70% of Tcm reg showed in 80-100% antileukemic functionality.
Conclusion: Higher proportions of different Treg subpopulations are found in AML patients compared to healthy probands and after DC-stimulation CD8+ Treg, Teff/em reg and CD39+ T-cells clearly correlate with reduced antileukemic activity. Refined analyses in the context of clinical responses to immunotherapies and GvH reactions are required.

## Multiple Myeloma

**P899**
**Allogeneic haematopoietic stem cell transplantation for multiple myeloma – the Swiss experience**
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The role of allogeneic stem cell transplantion (allo-HSCT) in the therapeutic armamentarium of multiple myeloma (MM) is very controversial. To assess the efficacy and safety of this procedure, we performed a retrospective analysis of all MM patients allografted in Switzerland.
Between October 1988 and February 2011, 94 MM patients received an allo-HSCT in the 3 national tranplant centers. Their median age at transplant was 47 (range, 24-66) years and the median interval from diagnosis to allo-HSCT was 22 (range, 3-156) months. 59 (63%) patients had IgG, 22 (23%) IgA, 5 (5%) IgD and 8 (9%) light chains MM. The median number of chemotherapy lines prior to allo-HSCT was 2 (range, 1-7). 40 (42%) patients were transplanted after achieving a first remission. 51 (54%) patients had been treated with novel drugs prior to allo-HSCT and 68 (72%) had received at least 1 autologous transplantation. 67 (71%) patients were transplanted with an HLA identical sibling donor. Conditioning regimen were non-myeloablative in 78% (n=64) of the patients. At time of allo-HSCT, 35 (37%) patients were in CR/VGPR, 35 (37%) in PR, 17 (18%) in SD and 9 (10%) in PD.
At time of data cut-off, the median follow up of survivors was 49 (5-233) months. Acute GVHD grade 2-4 and chronic extensive GVHD occurred in 46 (49%) and 42 (47%) patients, respectively. The cumulative incidence of transplant related mortality at 2 years was 17% (95% CI: 9%-25%). Median PFS was 430 days, with a 5 year PFS of 31% (95% CI: 22%-46%). Median OS was 5.7 years, with a 5 year OS of 51% (95% CI: 41%-61%). When comparing patients who received allo-HSCT in first remission versus in later stages of the disease, the former group had a significantly better 2 year OS (75% versus 60%, p=0.043) and PFS (65% versus 30%, p=0.007). Patients with chronic GvHD had an improved OS (HR 0.87, 95% CI: 0.45-1.72) and PFS (HR 0.88, 95% CI: 0.46-1.67), although this difference was not statistically significant. Finally, while PFS was not influenced by the type of donor, we observed an improved 2 year OS for patients with a related donor (74% versus 48%, p=0.007). We found no impact of novels drugs administration or conditioning regimen intensity on PFS and OS.
Although GVHD and transplant related mortaliy remain major limitations compromising the benefit of the graft-versus-myeloma effect, our series suggest that an early allo-HSCT in the disease course may provide the best results in a subset of selected patients.

**P900**

**Allografting from unrelated donors in multiple myeloma: a study by the Italian bone marrow donor registry**

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To evaluate the role of allografting from unrelated donors in the treatment of myeloma we conducted a study through the Italian Bone Marrow Donor Registry. Overall, from 2000 through 2009, 196 myeloma patients, median age 51 years (32-67), were transplanted at 34 Centers. Fifty-two (28.1%), 69 (37.3%), and 64 (34.6%) patients were treated with myeloablative, reduced-intensity and non-myeloablative conditioning respectively.

Incidence of acute grade II-IV graft-versus-host-disease (GVHD) was 46.4% whereas chronic GVHD was 45.1%. There was no difference in GVHD incidence among the 3 cohorts. Complete and partial remissions in patients who survived at least 3 months post-transplant were 27% and 28%. At a median follow up of 32 (0-118) months post-transplant, in the entire study population, median OS from diagnosis was 70.6 months while OS and EFS from the allograft were 18.9 and 14.9 months. Overall, the cumulative incidence of transplant related mortality (TRM) was 29.6% at 1 year and 32.4% at 5 years. OS from diagnosis and EFS from transplant were 70.6 and 28.2 months; 66.8 and 9.1 months; 111.9 and 22.4 months in patients who respectively underwent a myeloablative, a reduced-intensity and a non-myeloablative transplant. One-year and 5-year TRM was 33.3% and 35.7%, 32.2% and 34.4%, and 22.1% and 26.5% respectively. By univariate analysis, lower number of chemotherapy lines before the allograft, disease status at transplant, a fully HLA-identical donor, the use of peripheral hematopoietic cells rather than bone marrow were statistically significant variables for better OS whereas disease status at transplant, a fully HLA-identical donor, chronic GVHD (either limited or extensive) were statistically significant for better EFS. However, by multivariate analysis, only the development of chronic GVHD (HR 0.50; $p<0.001$) and a better response post-transplant (HR 2.11; $p<0.03$) were significantly associated with longer OS whereas chronic GVHD was the only variable associated with better EFS (HR 0.32; $p<0.001$). Acute GVHD was associated with both poorer OS (HR 2.35; $p<0.001$)) and EFS (HR 3.19; $p<0.001$). In conclusion there appears to be a strong association between chronic GVHD and graft-vs.-myeloma effects. However, long term disease control remains an issue regardless of the conditioning employed. Prospective trials may allow to define which patient category may most benefit from an unrelated donor allograft.

**P901**

**Allogeneic stem cell transplantation in patients with multiple myeloma: long term follow-up in a single centre**

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Background: Allogeneic stem cell transplantation (allo-SCT) is probably the only curative option for patients (pts) with multiple myeloma (MM). Here, we retrospectively studied a series of allo-SCT in 25 pts with MM in our center.

Patients and Methods: from April 1998 to december 2010, 25 pts with MM underwent allo-SCT with an HLA-identical sibling donor. Initially, 14 pts had MM with Ig G, 3 with IgA, 3 light chains, 3 non-secretory and two undetermined. Three pts were stage II and 22 stage III. At time of allo-SCT, 11 pts were in complete remission and 14 in refractory/progressive disease (4 received prior autologous transplant). Median age was 37 years (28–60) and the sex-ratio (M/F) 2,1. Median time from diagnosis to allo-SCT was 12 months (5–127). Two conditioning regimen used: Reduced intensity conditioning (RIC) with Fludarabine 150 mg/m$^2$ and Melphalan 140 mg/m$^2$ performed in 18 pts and Myeloablative conditioning regimen (MAC) with Busulfan 16 mg/kg associated with Cicophosphamide 120 mg/kg (7 pts). GVHD prophylaxis consisted on association cyclosporine (cSA)-mycophenolate (MMF) in RIC and CSA-methotrexate in MAC. All pts received G-CSF mobilised peripheral blood stem cells, with a median CD34+ cell count: 5,34.10$^6$/kg (1.9–13).

Results: Neutropenia occurred in all pts and the median duration of aplasia was 11 days (5–21). Only 10 pts (40%) required red blood cells transfusions and 21 pts (84%) needed platelets transfusions. Acute GVHD was observed in 12 cases (48%) including 10 cases of grade II–IV. Ten pts (58,8%) had chronic GVHD, with 7 an extensive form. Five pts (20%) had CMV reactivation. Seven pts (30,4%) relapsed at a median time of 13 months (8-45) with complete remission obtained in 3 pts (50%) after Donor Lymphocyte infusion with CD3 cells median dose: 7,5 10$^7$/kg. TRM was 40%. With a median follow-up of 110 months (17–137), 10 pts (40%) are still alive in complete remission with full donor chimerism. Fifteen pts (60 %) died (2 early severe infections, 6 GVHD, 4 after relapse, one sinusoidal obstruction syndrome, one myocardial infarction, and one public highway accident). Overall survival was 38,2% (40,6% in RIC and 33,3% in MAC) and progression-free survival was 24,4% (21,3% in RIC and 33,3% in MAC) respectively.

Conclusion: This study suggests that allo-SCT is a potential therapy for refractory MM. However, TRM and relapse remain a matter of concern. Place of HSCT in the era of novels drugs is still in discussion.

**[P900]**

| Conditioning | Myeloablative | Reduced-intensity | Non-myeloablative |
|---|---|---|---|
| Patient number (%) | 52/185 (28) | 69/185 (37) | 64/185 (35) |
| Median Age | 45 | 53 | 55 |
| Previous therapy lines < 2 (%) | 23 (27) | 33 (38) | 30 (35) |
| Previous therapy lines ≥ 2 (%) | 29 (29) | 36 (37) | 34 (34) |
| Stem Cell Source BM (%) | 24 (57) | 18 (43) | 0 (0) |
| Stem Cell Source PBSC (%) | 28 (19) | 51 (36) | 64 (45) |

**P902**

**Reduced-intensity allogeneic stem cell transplantation in myeloma: outcomes comparing high-risk disease treated early with multiply relapsed patients**

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The role of reduced intensity conditioning (RIC) allograft for myeloma remains controversial. Most studies have focussed on sibling allogeneic haematopoietic stem cell transplantation (allo-HSCT) in newly diagnosed patients following initial induction chemotherapy and autologous HSCT. Information is lacking on patients with adverse clinical features, such as poor response to initial treatment. Here we report the single-centre outcome of 19 patients with myeloma undergoing RIC allo-HSCT using a Fludarabine/TBI (2 Gy) based conditioning regimen. Median age at HSCT was 51 years. Patients were analysed in 2 groups; patients transplanted ≤ 18 months (median 10 months; range 7-18 months) from presentation with adverse clinical features (up-front group, n=9), and patients transplanted >18 months (median 44.5 months; range 26-123 months) from presentation (delayed group, n=10). Patients in the up-front group included plasma cell leukaemia (n=2), extramedullary disease (n=3) and poor responders to first-line chemotherapy/ autologous HSCT (n=2), whereas all patients in the delayed group had multiply relapsed disease. Median number of lines of chemotherapy in up-front *vs.* delayed groups were 2 *vs.* 6 respectively, with 18 patients having received ≥ 1 autograft. Patients in the up-front group were treated with tandem autograft/allograft (n=8), compared with only 2 patients in the delayed group. Donors were 10/10 HLA-matched unrelated donors (n=9), sibling donors (n=9), and 4/6 matched single cord (n=1). Median follow-up was 26 (4-47) months. All but one patient engrafted (cord transplant). Of the 9 patients in the up-front group, 6 achieved CR post allo-HSCT of whom 5 remain in CR, compared with 4 out of 10 patients in the delayed group achieving CR, of whom only one remains in CR. Of the remaining patients, there were 2 deaths (TRM n=1, relapse n=1) and one stable VGPR in the up-front group, compared with 4 deaths (TRM n=2, relapse n=2), 4 relapses and 1 stable PR in the delayed group. One patient in each group died from GvHD in CR. The incidence of acute GvHD was similar in both groups (33 *vs.* 30%), whereas chronic GvHD was less prominent in the up-front group (40 *vs.* 67%). Overall survival at 2 years was 60% [up-front group (74%); delayed group (40%)]. We conclude that RIC allo-HSCT for myeloma is effective therapy for patients with adverse risk disease treated early compared to patients with multiply relapsed myeloma.

**P903**

**Toxicity-reduced, myeloablative allograft followed by maintenance therapy as salvage therapy for refractory/ relapsed myeloma patients**

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We report the results of myeloablative toxicity-reduced allo-SCT after intravenous busulfan (12.2 mg/kg), cyclophosphamide (120 mg/kg) and antithymocyte globulin (ATG Fresenius®) (median 20 mg/kg day -3, -2, -1) followed by lenalidomide (lena) maintenance in 33 patients with multiple myeloma. Patients (pat.) had received one (n=16), two (n=15), or three (n=1) autografts, and 1 pat. was refractory to 2 induction therapies and failed to collect autologous stem cells. Primary endpoint was non-relapse mortality at 1 year and secondary objectives were disease response, incidence of acute and chronic graft-versus-host disease (GVHD), progression-free and overall survival. Nineteen patients were treated with fully HLA-matched, 8 a mismatch unrelated donor and 6 an HLA-identical sibling. Lena was started earliest 120 days after allo-SCT if there were no signs of infection or GVHD.
Nine pat. did not receive lena due to ongoing GVHD, cytopenia or patient's wish. Median number of lena cycles was

**[P902]**

| Patient no. | Sex | Age at HSCT (years) | Diagnosis | No. lines of chemo before RIC allo | Number of previous autos | Tandem/ non-tandem† | Time diagnosis to allo (m) | Donor | Disease status at start of RIC allo | Best disease status post RIC allo | GvHD | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 54 | IgG lambda myeloma | 6 | 1 | non-tandem | 35 | 10/10 matched MUD-PBSC | PR | vgPR | Extensive - gut GvHD | Death D+124 from relapsed disease |
| 2 | F | 53 | IgG kappa myeloma | 6 | 2 | tandem | 90 | HLA-id sib | PR | PR | Nil | Alive +37 months; 100% donor chimerism; DLI x6 for residual disease; stable pp 3g/L |
| 3 | F | 48 | Lambda LC myeloma | 5 | 1 | non-tandem | 33 | 10/10 matched MUD-PBSC | CR | CR | Extensive - liver, skin | Death +24 months from sepsis (in CR; off immunosuppression) |
| 4 | M | 56 | Lambda LC myeloma | 7 | 2 | non-tandem | 67 | 10/10 matched MUD-PBSC | vgPR | vgPR | Limited - skin, eyes | Death +17 months from relapsed disease |
| 5 | M | 56 | IgA kappa myeloma | 4 | 1 | non-tandem | 28 | HLA-id sib | vgPR | vgPR | Nil | Alive +28 months; 100% donor chimerism; DLI x2 for persistent disease; relapsed - responding to lenolidamide/dex; vertebroplasty for new vertebral lesion |
| 6 | M | 56 | IgA lambda myeloma | 6 | 2 | tandem | 38 | HLA-id sib | vgPR | CR | Limited - skin | Alive +16 months; 100% donor chimerism; relapsed - on lenolidamide/dex and planned DLI |
| 7 | F | 48 | IgG kappa myeloma | 10 | 2 | non-tandem | 123 | 10/10 matched MUD-BM | PR | PR | Limited - skin, oral | Alive +15 months; 100% donor chimerism; DLI x1 for residual disease; relapsed - currently responding to lenolidamide/dex |
| 8 | M | 45 | IgG kappa myeloma | 5 | 1 | non-tandem | 51 | 10/10 matched MUD-PBSC | PR | PR | Limited - gut GvHD | Alive D+223; relapsed. 88% donor chimerism; tapering immunosuppression |
| 9 | M | 58 | IgG kappa myeloma | 6 | 2 | non-tandem | 60 | HLA-id sib | CR | CR | Extensive - gut GvHD | Death D+83 from sepsis and gut GvHD |
| 10 | M | 51 | IgA kappa myeloma | 4 | 1 | non-tandem | 26 | 10/10 matched MUD-PBSC | CR | CR | Nil | Alive D+132; CR; 100% donor chimerism |

| Patient no. | Sex | Age at HSCT (years) | Diagnosis | No. lines of chemo before RIC allo | Number of previous autos | Tandem/ non-tandem† | Time diagnosis to allo (m) | Donor | Disease status at start of RIC allo | Best disease status post RIC allo | GvHD | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | M | 47 | IgG lambda MM | 3 | 1 | non-tandem | 12 | HLA-id sib | PR | CR | Limited GvHD | Alive +47 months; 100% donor chimerism; in CR with mild cGvHD |
| 12 | M | 44 | IgG lambda MM | 2 | 1 | tandem | 10 | HLA-id sib | CR | CR | Limited GvHD | Alive +39 months, in CR; 100% donor chimerism; GvHD resolved |
| 13 | F | 40 | IgA kappa MM | 2 | 1 | tandem | 8 | HLA-id sib | CR | CR | Extensive GvHD | Death +13 months from severe, extensive cGvHD |
| 14 | M | 44 | IgG kappa MM | 2 | 1 | tandem | 7 | 10/10 matched MUD-PBSC | PR | vgPR | Limited GvHD | Alive +30 months, in vgPR (pp too lowto quantitate); 100% donor chimerism; ongoing mild GvHD skin on immunosuppression |
| 15 | F | 50 | Plasma cell leukaemia | 3 | 1 | tandem | 8 | Cord 4/6 match | CR | CR | Nil | Alive +29 months; in CR with full autologous reconstitution (0% donor chimerism) |
| 16 | M | 50 | IgG lambda MM | 2 | 1 | tandem | 10 | HLA-id sib | CR | CR | Limited GvHD | Alive +26 months; in CR; 100% donor chimerism; on immunosuppression for cGvHD kidneys (nephrotic range proteinuria) |
| 17 | F | 63 | IgG kappa MM + plasma cell leukaemia | 2 | 0* | non-tandem | 7 | 10/10 matched MUD-PBSC | CR | CR | Limited GvHD | Alive +10 months; in CR; 100% donor chimerism; oral cGvHD on tapering immunosuppression |
| 18 | M | 59 | IgG kappa + lambda + IgA lambda | 3 | 1 | tandem | 18 | 10/10 matched MUD-PBSC | vgPR | Progressive disease | Nil | Death D+140 from progressive disease |
| 19 | M | 55 | LC lambda MM + mesenteric plasmacytoma | 3 | 1 | tandem | 10 | HLA-id sib | PR | CR | Nil | Alive D+202; in CR; 100% donor chimerism |

6 (1-30). Lena was discontinued due to progressive disease (n=6), GvHD (n=3), thrombocytopenia (n=2), or fatigue (n=2). In 10 patients lena dose could be increased to 10 or 15 mg, respectively. Major toxicities of lena were acute GVHD grade I–III (21%), viral reactivation (12%), thrombocytopenia grade III-IV (12%), neutropenia grade III-IV (6%), peripheral neuropathy grade I-II (12%), or other infectious complications (6%).

Two pat. died of treatment-related complications resulting in a cumulative incidence of non-relapse mortality at 1 year of 6% (95% CI: 0-14%). Acute GVHD II° and III° were seen in 27% and 6% of pat. respectively. Complete remission, partial remission and stable disease were seen in 46%, 48% and 3% of pat. respectively.

Nine patients experienced relapse resulting in a cumulative incidence of relapse at 3 years of 42% (95% CI: 18-66%). The 3 year estimated probability of progression-free and overall survival was 52% (95% CI: 28-76%) and 79% (95% CI: 63-95%), respectively. Neither deletion 13q14, mismatch donor nor chemosensitivity prior allo-SCT were prognostic for survival. This study showed that toxicity-reduced myeloablative conditioning regimen is feasible and highly effective in relapsed patients with multiple myeloma resulting in an acceptable treatment-related mortality. Lena maintenance therapy is feasible early after transplantation but toxicity especially the induction of graft-versus-host disease has to be considered.

**P904**

**Immunomodulatory effects of bortezomib / lenalidomide combination therapy as maintenance early after allogeneic stem cell transplantation for patients with multiple myeloma**

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We investigated the immune modulating effects of a combined application of bortezomib and lenalidomide as maintenance therapy for Multiple Myeloma patients after allografting. We treated 3 patients, starting between day 104 and 284 after allogeneic stem cell transplantation (SCT). Patients received 4 cycles of 5mg lenalidomide on days 1 to 21 of a 28-day schedule and 1 mg/m2 bortezomib once weekly for the same period. We measured NK, T, and B cell subset frequencies and function in peripheral blood samples. Interferon gamma (IFNg) producing T cells, especially TH1 cells, but not NK cells were significantly reduced (11% *vs.* 4.2%; $p<0.05$). Along with the TH1 cells, central memory CD4 T cells, showed a significant decrease after one month of treatment (4.3% *vs.* 0.4%; $p<0.05$). Furthermore, mature but not naïve T and B cells were inhibited by the lenalidomide/ bortezomib combination.

We did not observe a single case of GvHD, significant polyneuropathy, or haematotoxicity. However, virus infections were major adverse events. This may a direct consequence of the suppression of TH1- cells as well as mature B- and central memory T- cells. We conclude, that the combination of lenalidomide / bortezomib showed acceptable toxicity, but requires careful monitoring of viral infections. The use of anti-viral prophylaxis is strongly recommended.

**P905**

**High-dose chemotherapy with autologous stem cell transplantation for multiple myeloma: experience from a developing country**

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Objectives: Autologous peripheral blood stem cell transplantation is the therapy of choice for the treatment of multiple myeloma (MM) patients younger than 65 years old.

We analyzed the results of 61 patients (41 males and 20 females) with multiple myeloma who underwent autologous stem cell transplantation (ASCT).

Patients and Methods: The median age of patients was 55 years (range, 35–65 years). Before transplant, patients received chemotherapy using VAD (vincristine, adriamycin and dexamethasone, n=19) or VD ( Bortezomib and dexamethasone, n=22), and 20 patients received more than one regimen. The median CD34+ stem cells was $3{,}19 \times 10^6$/kg (1.22-9.70). High-dose melphalan (200 mg/m²) was used for conditioning.

Results: Before transplant, CR was 21% versus 54% versus 26% with VAD, VD, over one regimen respectively ($P \leq 0.05$). Median time to achieve more than $0.5 \times 10^9$/l granulocytes

was 10 days, whereas median time to recover above 20x109/l platelets was 13 days. After transplant, CR was achieved in 44 cases and a very good partial response in 6 cases. The overall response was 82% (CR plus VGPR) versus 66% before transplant (P≤0.05). The 100-day mortality was 1.6% (one patient). At a median follow-up of 13 months, the overall median post-transplant survival has not been reached, whereas the 31-month survival is 93%±0.05% (27,72-31,16).
Conclusion: Survival results were substantially better than those of historical control in a group of patients treated in the same institution without ASCT.

**P906**

**Bortezomib-based treatment followed by autologous transplant in multiple myeloma. A single-centre experience**

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In the last years Bortezomib Treatment (BT) has demonstrated an high response rate in Multiple Myeloma, both in first-line and in relapsed/refractory patients (pts).
We present a group of 44 pts who have been treated in the last five years with Bortezomib based regimens in first line (25 pts) or after failure of the first-line treatment to achieve a Complete Remission or Very Good Partial Remission (CR/VGPR) (19 pts). After BT, all these pts have been submitted to single or double Autologous Hemopoietic Stem Cell Transplantation (AHSCT) as consolidation program. Pts who received BT at diagnosis had advanced disease or adverse prognostic factors.
Aim of the Study: Primary end points were the response rate after BT and after AHSCT, the Progression Free Survival (PFS) and the Overall Survivall (OS). Secondary end points were incidence of severe complication in AHSCT and the difference between pts treated in first and second line.
Results: 29/44 (65%) pts obtained a CR/VGPR after BT, 14/44 (32%) pts had partial remission (PR) and only 1 pt showed refractory disease. CR/VGPR was observed in 20/25 (80%) and 9/19 (47%) pts treated in first and in second line respectively. After AHSCT 37/44 (83%) pts were in CR/VGPR and only 7/44 (14%) pts remained in PR.
Relapse after AHSCT were observed in16/44 (36%) pts. 15 of them were in CR/VGPR and 1 in PR after AHSCT. 7 pts received BT in first line and 9 pts in second line. 2 pts have been submitted to Allogeneic Transplant: one who relapsed and one with PR after AHSCT. 7/44 (16%) pts died, 6 for progression of disease and 1 for transplant related mortality (TRM) after allogeneic transplant. Median PFS from AHSCT is 24 months (median OS not yet reached) with a median follow-up of 22 months.
TRM after AHSCT is 0/44, we observed 2 cases of life threatening infections (pneumonia), and 3 CMV reactivations. No significant differences appeared between pts treated in first or in second line with BT in terms of PFS and OS.
Conclusion: BT seems to be very efficient in order to obtain a CR/VGPR also in pts who did not responded or partially responded to other treatment. HSCT can improve the response obtained after BT. Despite these results 15/37 (40%) pts who achieved a CR/VGPR had a progression of the disease. These data suggest to explore which is the role of the AHSCT as consolidation treatment and to evaluate the strategy to maintain the CR/VGPR after its obtaining.

**P907**

**Lenalidomide after autologous stem cell transplantation in multiple myeloma**

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Introduction: The prognosis of patients that are suffering from multiple myeloma (MM) which process is progressing after autologous hematopoietic stem cell transplantation (AHSCT) is poor. However, in recent years major advances have occurred with the addition of immunomodulatory drugs such as lenalidomide. It is used as a rescue therapy or as a maintenance one. Both of them improve the survival of these patients.
Objective: The objective of this study was to analyze retrospectively the rate of responses and safety in patients suffering from multiple myeloma treated with lenalidomide after AHSCT.
Methods: 22 patients undergoing ASCT between 2000 and 2010 were evaluated. They received lenalidomide 25 mg/day for 21 days every month and dexamethasone 40 mg on days 1-4 of the cycle. Nineteen patients were started on lenalidomide because of the progression of the disease, while three ones were started as the maintenance therapy because of their age (<40 years). All patients received antithrombotic prophylaxis with aspirin 100 mg/d.
Results: Twenty-two patients were analyzed, 40% women and 60% male. The median age was 64 years (32-68). Pre AHSCT induction treatment consisted on receiving VAD (n=14), VBCMP/VBAD (n=2), Bortezomib/dexamethasone (n=4) and dexamethasone alone (n=2). As for the post ASCT evaluation, 15 patients reached a partial response (PR); three, a very good partial response (VGPR); two, a complete response (CR); 1, the stable disease (SD) and 1, the progressive disease. Treatment toxicity was primarily hematologic (45%) with grade 3-4 neutropenia in 22% and 2-3 grade thrombocytopenia in 18%, forcing doses reduction in 3 cases.
After a follow-up median since the beginning of lenalidomide application for 18 months (3-37), all patients achieved any degree of response (6CR, 6VGPR, 10PR), and 45% improved the post AHSCT response. Six patients had started the maintenance treatment with thalidomide after transplantation but they were switched to lenalidomide because of the toxicity. During the follow-up, 5 patients (23%) have progressed with an average time to progression of 10.8 months. Three have died from disease progression. Overall the survival after a follow-up average from diagnosis of 48 months is 86.3%.
Conclusions: In our experience, lenalidomide is safe and effective treatment while it maintains and even improves the responses obtained after transplantation in multiple myeloma disease.

**P908**

**Intravenous busulfan and melphalan as conditioning regimen for autologous stem cell transplantation in patients with newly diagnosed multiple myeloma: a matched comparison to a melphalan-based approach**

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Melphalan 200 mg/m$^2$ (MEL200) is the standard conditioning regimen administered to newly diagnosed patients with multiple

myeloma (MM) undergoing autologous stem cell transplantation (ASCT). In fact, few alternatives have been explored in order to improve the antimyeloma activity of this conditioning. Here we compare intravenous (iv) busulfan (BU) 9.6 mg/kg and melphalan (MEL) 140 mg/m$^2$ (BUMEL) versus melphalan, MEL200 as conditioning regimen before ASCT for newly diagnosed patients with multiple myeloma. For this purpose, 51 patients receiving BUMEL undergoing ASCT between 2005 and 2009 were compared in a 1:2 matched control analysis to 102 patients receiving MEL200 transplanted from 2001 to 2005 (control group). Patients in the control group were included in the PETHEMA (Programa Español de Tratamientos en Hematología)/GEM2000 study. Matching criteria included age, clinical stage at diagnosis, and response to induction therapy. No differences in the overall and complete response rates were observed after ASCT between both groups. After a median follow-up of 63 and 37 months in control and BUMEL groups, progression-free survival (PFS) was 24 and 38 months, respectively. The 5-year PFS was 22% (95% CI: 18–26%) in the control group compared to 35% (95% CI: 27–43%) in the BUMEL (P=0.1). Time to progression was 26 and 39 months in the control and BUMEL groups, correspondingly (P=0.1). Hematopoietic reconstitution was similar in the two groups and the toxicities most frequently observed included mucositis and febrile neutropenia in both groups. Seven (14%) out of the 51 patients conditioned with BUMEL developed grade I/II liver toxicity that did not require any specific therapy. Interestingly, however no case of sinusoidal obstruction syndrome was observed in the BUMEL cohort. Transplant-related mortality was 4% and 2% in BUMEL and control groups, respectively. ASCT conditioned with iv BUMEL may be considered an effective and well-tolerated alternative to a MEL200 transplant-based approach as conditioning regimen for MM patients who are candidates for ASCT.

**P909**

**Comparison of high-dose melphalan with or without bortezomib as conditioning regimen for autologous stem cell transplantation in newly diagnosed multiple myeloma patients beyond very good partial response after bortezomib-based induction therapy**

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Objectives: High-dose melphalan (HDM, 200 mg/m$^2$) in combination with autologous stem cell transplantation (ASCT) is the standard treatment for young patients with multiple myeloma (MM). To assessed the role of bortezomib (Bor) in conditioning regimen by comparing efficacy and safety of HDM (Bor-HDM) with or without bortezomib as conditioning regimen in newly diagnosed MM patients who had achieved very good partial response (VGPR) or complete response (CR) after bortezomib-based induction therapy.
Methods: Twenty two patients were enrolled from February 2008 to March 2010, and Bor-HDM and HDM regimens before ASCT were administered to 10 and 12 patients, respectively.
Results: No marked differences in oral mucositis, gastrointestinal symptoms (nausea, vomiting and diarrhea), newly occurring symptoms of nervous system, duration of neutropenia, and incidence of fever were observed between the two groups. No transplantation-related mortality was observed. Both groups had similar myeloid and platelet engraftment after ASCT. Response of ASCT was comparable for two group. The median time to progression of disease in Bor-HDM group and HDM group were 20 months and 22 months, respectively. The 2-year progression-free survival (PFS) rate was 41.1% for Bor-HDM group and 48.6% for HDM group. No significant difference in the disease-progression-free rate was observed between groups (p=0.771).
Conclusion: These results suggest that, the benefit of further combination bortezomib with HDM in conditioning regimen was limited for newly diagnosed MM patients with VGPR or CR subgroup after bortezomib-based induction therapy.

**P910**

**Autologous peripheral blood haematopoietic stem cell transplantation for multiple myeloma, experience in southern Iran**

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Hematologic malignancy currently represents the main indication for HSCT. Clearly, autologous and allogeneic HSCT are established therapies in many of hematologic malignancies. High dose therapy (HDT) supported by autologous HSCT are the preferred choice for lymphoproliferative disorders and multiple myeloma. Several clinical trials have shown the superiority of high-dose therapy (HDT) and autologous stem cell transplantation (ASCT) over conventional dose therapy for patients with multiple myeloma.
During 7 years, we treated 57 patients with multiple myeloma (mean age=50, range: 31-70) with a preparative regimen of Melphalan 140–200 mg/m$^2$. Patients were treated by intensive chemotherapy followed by reinfusion of non- cryopreserved autologous stem cells. The source of stem cell in all patients was peripheral blood.
The median time of hospitalization was 21 days (range: 16-36). The median time to platelet count >20×10$^9$/L was 14 days (range: 10–33). Also, the median time to absolute neutrophil count >0.5 × 10$^9$/L was 11 days (range: 9–21). All the 57 patients were engrafted and there was not graft failure in this study group. Responses (complete and partial response) were seen in all the 57 patients. One hundred days transplant-related mortality was 1.7%.
We concluded HDT and autologous stem cell transplantation without cryopreservation is an effective and safe method which simplifies the procedure and is feasible and cost saving in our patients.

**P911**

**Transplantation activity in NSBALHZ (CIC 859)**

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Since 2004 year a total of 191 eligible patients are treated with autologous (ASCT) and allogeneic hematopoietic stem cell transplantation (allo-SCT) as a standard therapeutic procedure according to the universally accepted guidelines in Bone Marrow Transplantation ward at NSBALHZ, Sofia, Bulgaria (CIC 859).
Aim: The aim of the study was to analyze the outcome, including overall survival (OS), progression-free survival (PFS), relapse rate (RR) and transplanted related mortality (TRM) for the procedure of transplanted patients at NSBALHZ during the period of 2004 to July 2011 year. The response assessment post-transplantation was defined as: complete remission (CR), partial remission (PR) and no answer/progression (PROG) rates.
Patients and Methods: 184 patients received auto-SCT were included. The auto-SCT group comprised of multiple myeloma patients (MM, n=78), Morbus Hodgkin (MH, n=52), non-Hodgkin lymphoma (NHL, n=44), acute leukemia (AL) and solid tumour (n=3). Due to the small number allogeneic transplanted patients, this group was excluded from the analysis.
Results: In all ASCT were used myeloablative conditioning regimen according to the patient's diagnosis and peripheral blood stem cell (PBSC). In NHL auto-transplanted patients group were registrated CR rate of 48%, PR 9%, RR 2%, PROG 18%, TRM

5%. The 3-year OS was 63% and the 3-year PFS 49%. MH group after ASCT CR was 40%, PR 15.4%, RR 0%, PROG 34.6% and TRM 5.8%. OS and PFS were respectively 54% and 46%. In myeloma patients CR was 19.6%, VGPR 56%, PR 21.3%, PROG 0% and TRM 3%. OS 85.6% and PFS 63.1%. Due to ASCT 85.6% of AL patients attained CR on 3th month. Nowadays only 1 patient is still alive in CR and the other died because of relapse. Conclusion: The presented results of our eight-year experience in the HSCT field show that transplantation of hematopoietic stem cell now is a preferable method of treatment in eligible patients with high risk and relapsed oncohematological malignancies. Unfortunately the problem of late decision sending patients for ASCT and the professional understanding of the role of stem cell transplantation still exist. This is why our future efforts are aimed at increasing transplanted patients number and incorporating HLA incompatible unrelated donor and HLA haploidentical sibling donor in routine clinical practice.

**P912**

**High-dose melphalan and aulologous stem cell transplantation in AL amyloidosis is a safe procedure leading to an improved overall survival in a single-centre study**

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High dose melphalan followed by autologous stem cell transplantation (HDM/ASCT) was investigated in AL amyloidosis based on its success in treating multiple myeloma patients. A complete hematologic response has been reported up to 72% of pts. The greatest matter is the transplant related mortality (TRM) which ranges from 5% to 14% in single centre studies and from 24% to 43% in multicenter studies. We report the results of a single centre study performed at BMT Unit in Pavia, from 2000 to 2010. Nineteen pts with amyloidosis received HDM rescued by ASCT. The median age of pts was 53 years (range 38-60), 8 male and 11 female. The median interval from diagnosis to ASCT was 277 days (range 133-1900). In all but one pt peripheral hemopoietic stem cells (PHSC) were harvested by one or two leukapheresis after mobilization with G-CSF subcutaneously at the dose of 10 mcg/kg for 4 or 5 days. In one pt (UPN 1132) PHSC mobilization was performed by intermediate dose of cyclophosphamide and G-CSF. The majority of pts had ASCT with active disease, only one (UPN 1076) received ASCT in complete remission. Three pts with heart failure had cardiac transplantation followed by HDM and ASCT. As far as the dose of melphalan, 6 of 19 received a melphalan reduced dose (140-160 mg/m$^{2)}$. All pts, in the attempt to reduce the risk of arrhythmias or sudden death, had washed stem cells: DMSO was removed by centrifugation, performed immediately prior to infusion. The hemopoietic recovery was documented at day + 13 (range +11-+42). At the time of the present evaluation 17 of 19 pts are alive, one pt died of transplant related complication at day +6, another pt died of disease progression at day + 190. Complete remission was achieved in 9 pts. The actuarial probability of survival at 5 years is 84% with a median survival of 934 days (range 190-4430). The progression free survival is 45% at 5 years. Eight pts with persistent clonal disease after ASCT received further treatment with either thalidomide and dexamethasone or bortezomib or dexamethasone alone. The low TRM (5%) and the long survival suggest that this approach is safe and lead to an improved overall survival.

**P913**

**Influence of stem cell mobilisation after cyclophosphamide, thalidomide and dexamethasone regimen in patients with newly diagnosed multiple myeloma**

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Background: CTD regimen has been known as an effective induction therapy in patients with newly diagnosed MM. But, there were inconsistent results for the autologous stem cell yield for transplantation. The aim of present study was to identify the influence of CTD therapy on outcome of peripheral blood stem cell (PBSC) collection.
Methods: Forty-eight patients received 4 cycles of CTD therapy. Stem cells were mobilized with cyclophosphamide (3.0 g/m$^2$) and G-CSF (10 μg/kg, daily) or G-CSF alone. Patients failing to collect ≤ 4.0 x $10^6$ CD34+ cells /kg received a second mobilization courses.
Results: The median age at diagnosis was 56 years (range, 39-69). Median duration from start of CTD therapy to first collection was 4.6 months (range, 3.3-8.7). Forty-four patients were mobilized with cyclophosphamide following with G-CSF and 4 patients with G-CSF alone. The median day of apheresis was 3 days (range, 2-7). The response rate for CTD regimen at mobilization was 10% (5/48) of CR, 25% (12/48) of VGPR and 63% (30/48) of PR. A median number of harvested CD34+ cells was 8.6 x $10^6$ cells/kg. At the first mobilization, 83% (40/48) of patients had been reached the minimal PBSC collection target of ≥2.0 x $10^6$ CD34+ cells/kg and 71% (34/48) of patients achieved the collection ≥4.0 x $10^6$ CD34+ cells/kg. At the end of second mobilization, 90%(43/48) of patients had yields of at least ≥2.0 x $10^6$ CD34+ cells/kg and 77% (37/48) of patients had yields of ≥4.0 x 106 CD34+ cells/kg. During mobilization period, three patients were developed grade 3/4 non-hematologic adverse events.
Conclusion: CTD regimen is an effective induction therapy in patients with newly diagnosed MM showing high response rate and acceptable rate of autologuos stem cell yield without any detrimental effect for the following stem cell collection.

**P914**

**Growth factor plus preemptive ('just-in-time') plerixafor successfully mobilises haematopoietic stem cells (HSC) in all multiple myeloma patients despite prior lenalidomide exposure**

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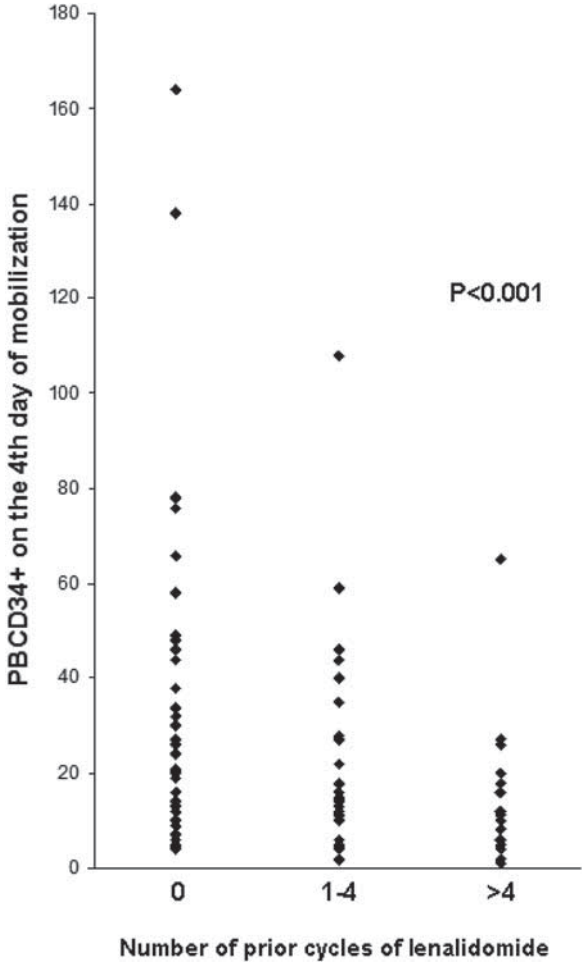
Objective: Lenalidomide, a component of several modern induction regimens in multiple myeloma (MM), leads to suboptimal autologous hematopoietic stem cell (AHSC) mobilization. Cyclophosphamide + filgrastim mobilization at least partially overcomes the negative impact of lenalidomide but leads to higher cost and toxicity. We hypothesized that growth factor + preemptive plerixafor is a safe and highly efficient strategy for initial AHSC mobilization in MM despite prior exposure to lenalidomide.
Methods: We retrospectively reviewed patient characteristics and mobilization outcomes of 89 consecutive MM patients undergoing first steady state mobilization with filgrastim or pegfilgrastim ± preemptive plerixafor using a previously validated algorithm based on day 4 peripheral blood CD34+ cell count (PB-CD34+) and mobilization target. Mobilization outcomes were analyzed for three distinct groups according to the extent of prior exposure to lenalidomide: no prior exposure (group A,

N=40), 1 to 4 cycles (group B, N=30) and > 4 cycles (group C, N=19).
Results: Median age was 57.5 years and similar across groups. Median number of prior lines of therapy was 1 in A and B and 2 in C. Median PB-CD34+ was 27/mm$^3$, 14.5/mm$^3$ and 10/mm$^3$ for groups A, B and C, respectively ( Figure 1, P<0.001). Multivariate analysis with PB-CD34+ as the dependent variable yielded only age, number of prior cycles of lenalidomide and mobilization with pegfilgrastim as significantly associated with PB-CD34+. Only 45% of patients in A required plerixafor *vs.* 63% in B and 84% in C, P=0.01. The median yield of CD34+ collected was 8.1 x 10$^6$ CD34+/kg in A, 7.4 x 10$^6$ CD34+/kg in B and 7 x 10$^6$ CD34+/kg in C. A higher proportion of patients in A (100%) met the mobilization target (6 x 10$^6$ CD34+/kg for most patients) than in B (90%) or C (79%), P=0.008. All patients collect at least 2 x 10$^6$ CD34+/kg. HSC collection was completed after a median of 1.5 daily apheresis sessions in A and B and 2 in C. The estimated cost of mobilization and collection was similar between A (median US$ 22,280) and B (US$ 22,280), but substantially higher in C (US$ 35,020).
Conclusion: Steady state growth factor mobilization with a validated algorithm for preemptive use of plerixafor is an adequate upfront mobilization strategy for MM patients regardless of prior exposure to lenalidomide negating the need for chemotherapy mobilization.




**P915**

**The stem cell mobilising potential of vinorelbine chemotherapy in myeloma patients is more efficient with G-CSF than with plerixafor (PAV-trial)**

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Background: Vinorelbine chemotherapy in combination with G-CSF is the standard regimen to mobilize autologous stem cells in myeloma patients in Switzerland. The potential of the CXCR4 inhibitor plerixafor is unclear within this concept. In addition, the stem cell mobilizing capacity of plerixafor together with chemotherapy, in the absence of G-CSF, is unknown in myeloma patients.
Methods: In this single arm phase II study, we analyzed 4 consecutive cohorts of 10 myeloma patients undergoing peripheral stem cell collection before high-dose chemotherapy in first remission. All 40 patients received 240 µg/kg plerixafor (P) at stem cell collection on day 8 (and, if necessary, day 9). In cohort 1 (VGP), patients received vinorelbine (V) 35 mg/m$^2$ on day 1 and G-CSF (G) 1 Mio U/kg/day starting at day 4, whereas no G-CSF was applied in cohort 2 (VP). In cohort 3, G-CSF was given, but not vinorelbine chemotherapy (GP). Finally, patients in cohort 4 received no G-CSF, but vinorelbine and delayed plerixafor (VPd) at the first day of a CD34+ increase exceeding 15,000 CD34+ cells/ml, (but not later than day 12). Results were compared to a historical control (68 patients) of our standard VG regimen with a median CD34+ mobilization of 99,120 CD34+ cells/ml. Plerixafor was given i.v. for 30 minutes at 8AM and apheresis started at 12PM to test stem cell collection in a one day procedure.
Results: We observed excellent stem cell mobilization (median 174,800 CD34+/ml) using the triple regimen VGP, whereas VP (cohort 2) resulted in a poor stem cell mobilization of only 9,390 CD34+ cells including one collection failure. Omitting vinorelbine in cohort 3 (GP) allowed stem cell mobilization (102,000 CD34+/ml) similar to our VG standard regimen. Individualizing the day of apheresis in cohort 4 (VPd), while omitting G-CSF, did not improve the inferior mobilization of VP (median 15,200 CD34+/ml). The i.v. plerixafor administration was well tolerated and allowed collection of stem cells in cohorts 1 (VGP) and 3 (GP) in a one day procedure in all patients.
Conclusion: Our results indicate that plerixafor can be safely added to mobilization chemotherapy with vinorelbine. Plerixafor and chemotherapy in the absence of G-CSF have a poor mobilization potential, whereas plerixafor together with G-CSF can replace vinorelbine chemotherapy allowing potent stem cell mobilization. Intravenously administered plerixafor (VGP or GP) offers convenient stem cell collection in a one day procedure.

**P916**

**High levels of peripheral circulating CD34+ cells at autologous stem cell collection are associated with favourable prognosis in multiple myeloma**

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Background: A variety of factors have been reported to affect prognosis in patients with multiple myeloma including cytogenetic abnormalities, molecular markers, cytokine profiling, and clinical parameters. In contrast, markers are widely lacking to characterize patients who preferentially benefit from high-dose chemotherapy with autologous stem cell transplantation which represents a cornerstone in the first-line treatment of multiple myeloma patients. Therefore, we investigated whether varying levels of mobilized CD34+ cells confer prognostic information in myeloma patients undergoing high dose chemotherapy.
Methods: In this retrospective study, we determined circulating CD34+ cells at the day of peripheral stem cell collection in 158 consecutive myeloma patients between January 2001 and August 2010 in a single academic center. Patients were

stratified into two groups (super versus normal mobilizers) with a cut-off of 100,000 peripheral CD34+ cells/ml. We hypothesized that excellent stem cell mobilization is associated with an intact bone marrow homeostasis and thus confers favorable prognostic information.
Results: We found that patients with more than 100,000 circulating CD34+ cells/ml peripheral blood (69 pts.) had a better overall survival (P=.005) and a prolonged time to progression (P=.0398) than patients with CD34+ cell counts below 100,000 CD34+ cells/ml (89 pts.). After a median follow-up of 32.5 months, 12 deaths occurred in the super mobilizer and 30 in the normal mobilizer group (P=.0289). Whereas the group of super mobilizers did not yet reach the median survival, the group of normal mobilizers had a median survival of 50 months. Patient characteristics did not differ between the two groups. High levels of CD34+ cells were an independent marker for better overall survival and time to progression in a multivariate analysis that included disease stage, response at transplant, light chain subtype, age, sex, and height.
Conclusion: Our results suggest that high levels of mobilized peripheral CD34+ cells are associated with favorable outcome in myeloma patients undergoing autologous transplantation. We propose that this biomarker might be considered to be integrated into future risk stratification in myeloma patients to select patients for a post-transplant maintenance or consolidation strategy.

**P917**
**The efficacy of blood progenitor stem cell mobilisation depending on the terms of G-CSF initiation in patients with multiple myeloma**
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Background: Cyclophosphomide (CY) combined with granulocyte colony-stimulating factor (G-CSF) is commonly used to mobilize blood progenitor stem cells to support high-dose therapy with melphalan in patients (pts) with multiple myeloma (MM).
Aim: To define the optimal terms of G-CSF initiation in patients with MM receiving high-dose CY for blood progenitor stem cell mobilization.
Methods: We have analyzed stem cell mobilization efficacy in 59 pts with newly diagnosed MM previously treated with VAD or bortezomib-containing courses in the department of highly-dose Chemotherapy and Bone Marrow Transplantation from June 2000 to December 2008. There were 32 male, and 27 female, median age 51,5 (range 36-68). In mobilization procedure pts received CY 6 g/m$^2$ followed by daily administration of G-CSF 5 mcg/kg. G-CSF was prescribed on day +3 after CY in 28 cases (first group), on day +5 in 7 cases (second group) and in 24 cases (third group) after the reduction in the number of WBC <1x10$^6$/l (+6-+9 day after CY, median +8 day).
Results: The date of the first apheresis procedure did not differed in three groups and was on the day +15 after CY administration. The longitude of G-CSF administration was 11-20 (median 14) days in the first group, 9-15 (median 12) days in the second group and 6-13 (median 9) days in the third group (p<0,05). The median number of aphereses performed in each group was 2 (range 1-5).
The regimens proved to be effective in the progenitor cell mobilization. In the first group 31,4 (1,1-93,8)x10$^6$ CD34+ cells/kg were collected, in the second group the number of CD34+ cells was 29,2 (2,06-100)x10$^6$/kg and in the third group – 31,2 (5,5-106)x10$^6$ CD34+ cells/kg.
The terms of G-CSF administration did not influence on the highest yield of peripheral blood (PB) CD34+ cells. The highest yield of PB CD34+ cells was 326740±80728 cells/ml in the first group, 335391±64505 cells/ml in the second group and 311043±39678 cells/ml in the third group.
Conclusion: The date of the first leukapheresis was not in dependence on the terms of G-CSF initiation. The efficacy of stem cell mobilization was the same in 3 groups of pts in spite of the term of G-CSF initiation or the longitude of its administration. As the analysis showed that economy of 3-5 doses of G-CSF due to the later administration of drug never reduce the efficacy of stem cell mobilization leaving to the collection quantity of CD34+ cells that is enough for double autologous stem cell transplantation.

**P918**
**Comparison of cytotoxic effects of antithymocyte globulins and antimyeloma globulins**
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Introduction: Antithymocyte globulines (ATG) are widely used in allogeneic stem cell transplantation for prophylaxis of graft-versus-host disease and known to induce cytotoxicity against various human cells. Preclinical studies have reported ATG-induced apoptosis and complement-dependent cytotoxicity in human myeloma cells *in vitro* and in a mouse model. Antibody-based treatment for myeloma has been unsuccessful in part due to the heterogynous phenotype of myeloma cells.
Methods: In an effort to improve the anti-myeloma effect and reduce toxicity of polyclonal antibodies, antimyeloma globulins (AMG) were produced by immunizing rabbits with myeloma cell lines RPMI-8226 (AMG-8226) or KMS12BM (AMG-12BM) and purified (Fresenius Biotech) using the same procedure as for ATG. Cytotoxicity of ATG-Fresenius and the AMGs was compared in primary T cells, myeloma cells (RPMI-8226, KMS12-BM, OPM-2) and non-haematopoietic cell (Hacat and Panc1). NK cell mediated antibody-dependent cell cytotoxicity (ADCC) was studied using chromium release assay, while other cytotoxicity assays were analysed by flow cytometry after staining with 7AAD. Combination effects of the polyclonals and bortezomib or melphalan were analysed using Calcusyn®.
Results: AMGs demonstrated stronger cytotoxicity against myeloma cells and T cells compared to ATG. Cytotoxicity against non-haematopoietic cell lines tended to be higher for ATG compared to both AMGs. AMG-8266 was used for further comparisons. Competitive blocking assays revealed that AMG-8266 contained 4x more specific antibodies against CD38 compared to ATG. At high concentrations of 100-500 ug/ml both ATG and AMG inhibited NK mediated ADCC ($p<0.001$ in both cases) compared to control with only NK cells. ATG thereby inhibited ADCC significantly more than AMG-8266 ($p<0.001$). Both polyclonals augmented NK cytotoxicity in low concentrations of 0.1-10 ug/ml ($p<0.001$ in both cases) compared to NK cells alone.
Combinations of ATG with melphalan or bortezomib showed synergistic cytotoxic effects on KMS-12BM cells with combination effects (CI) ranging from 0.69 to 0.94 and 0.69 to 1.10 respectively. Combination of AMG with melphalan and bortezomib showed similar synergistic effects with CI 0.70 to 0.98 and 0.70 to 1.0 respectively.
Conclusion: Our data show favorable anti-myeloma effects of AMG compared to ATG, toxicity however remains considerable. There is still need for development of more specific antibodies for the treatment of myeloma.

[P918]

| Table 1: complement-dependent cytotoxicity of ATG vs. AMG in myeloma cell lines, primary T cells, Hacat and Panc1 cell lines. | | | |
|---|---|---|---|
| KMS-12-BM | AMG-8226 vs. ATG | p = 0,00029 | n = 20 |
| | AMG-12-BM vs. ATG | p = 0,00020 | n = 20 |
| | AMG-12-BM vs. AMG-8226 | p = 0,01304 | n = 20 |
| | | | |
| RPMI-8226 | ATG vs. AMG-8226 | p = 0,00089 | n = 20 |
| | ATG vs. AMG-8226 | p = 0,01347 | n = 20 |
| | AMG-8226 vs. AMG-12-BM | p = 0,00029 | n = 20 |
| | | | |
| OPM-2 | ATG vs. AMG-8226 | p = 0,00029 | n = 20 |
| | ATG vs. AMG-12-BM | p = 0,00054 | n = 20 |
| | AMG-8226 vs. AMG-12-BM | p = 0,00451 | n = 20 |
| | | | |
| Primary T cells | AMG-8226 vs. ATG | p = 0,00009 | n = 20 |
| | | | |
| Hacat | ATG vs. AMG-12BM | p = 0,07474 | n = 6 |
| | ATG vs. AMG-8226 | p = 0,24886 | n = 6 |
| | AMG-12-BM vs. AMG-8226 | p = 0,04640 | n = 6 |
| | | | |
| Panc1 | ATG vs. AMG-12BM | p = 0,05802 | n = 10 |
| | ATG vs. AMG-8226 | p = 0,00691 | n = 10 |
| | AMG-12-BM vs. AMG-8226 | p = 0,00769 | n = 10 |

**P919**

**T-cell large granular lymphocyte leukaemia post successful auto-SCT in multiple myeloma: origin from coexisting undetected small-sized clone of neoplastic T-cells?**

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Background: Post-transplant lymphoproliferative disorders (PTLDs) were initially recognized in solid organ transplant recipients where its incidence can range from 1% to 6%. Among patients undergoing haematopoietic cell transplantation, PTLDs occur almost exclusively in recipients of allogeneic grafts with an overall incidence rate of 1% to 2% and manifest early post-transplantation within the first years. PTLDs are extremely rare in patients undergoing auto-SCT and the median time from diagnosis of MM is 56 months (Fenk 2011).
Case Report: We report the case of a 70 year-old male patient underwent a first auto-SCT for MM who manifested early PTLD. He was conditioned with melphalan 140 mg/m$^2$; on day 0, he received cryopreserved peripheral blood stem cell (4x106 CD34+ cells/kg). Engraftment was prompt, neutrophil recovery > 0,5 x 10$^9$/l and platelets > 20x10$^9$/l occurring on days +11 and +13, respectively. Surprisingly, on day +43 from auto-SCT, peripheral blood smear showed absolute lymphocytosis (WBC count 14.9x10$^9$ cells/L; absolute lymphocytic count 9.5x10$^9$ cells/L). The bone marrow aspirate and bone marrow trephine biopsy confirmed the presence of a population of large granular lymphocytes, mimicking a chronic lymphoproliferative disorder. Immunohistochemistry and flow cytometry immunophenotyping, performed on fresh cells obtained both from bone marrow and peripheral blood, showed T-cell-associated antigens (CD3+, CD4-, CD8+, CD56-). Sierological tests for hepatitis C and B virus, CMV, EBV, Parvovirus B19, Toxoplasma gondii and HIV were negative. Monoclonal T-cell receptor rearrangement was the most sensitive and appropriate method to detect clonality in T cells; immunoglobulin heavy and light chain genes were not rearranged. On FDG-TC/PET no lymphoadenopathy and splenomegaly were observed.
Conclusions: T-LGL leukemia has been seen after solid organ transplantation and allo-SCT; the PTLDs are extremely rare in auto-SCT patients. In allo/auto-SCT, EBV serologic status and viral infections represent a pathologic state in which chronic antigenic stimulation may result in T-cell clonal expansions; in addition, incidence of PTLDs is related to intensity of immunosuppression and additional genotoxic stress of therapeutic programs. In our case, both the negativity of all viral serological tests and very short time interval of occurrence, we push to hypothesize that T-LGL leukemia derives from coexisting undetected small-sized clone of T-cells.

**P920**

**Significant improvement of neuropathy after autologous stem cell transplantation in POEMS syndrome**

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Background: POEMS syndrome includes Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal gammopathy and Skin changes. POEMS is a rare, rapidly evolving and devastating multisystem disease. Roughly 60 patients treated with high dose chemotherapy and autologous stem cell transplantation (ASCT) have been reported so far. A higher rate of engraftment syndrome and excessive morbidity compared to ASCT used in

other diseases were observed in small series of POEMS syndrome patients undergoing ASCT.
Methods: We here report a 58 years old male patient with POEMS syndrome treated with ASCT at our institution. Rapidly progressive polyneuropathy ultimately evolving to tetraparesis and paresis of the diaphragm, associated with monoclonal gammopathy (type IgG lambda), mild hepatosplenomegaly, multiple endocrine abnormalities and sclerotic bone lesions led to the diagnosis of POEMS syndrome. Typically, VEGF levels were elevated. The patient was bedridden and required noninvasive ventilation support. The cardiac situation was complicated by a sino-atrial and a complete atrio-ventricular block requiring implantation of a pacemaker.
Results: Peripheral blood stem cells were mobilized with granulocyte-colony stimulating factor without chemotherapy. High-dose melphalan (200 mg/m$^2$) was used as conditioning regimen. Early post-transplant complications included neutropenic fever, septic shock and respiratory failure, necessitating temporary treatment in the intensive care unit. After hematologic recovery, continuous neurologic improvement was observed. Six months after ASCT, the patient was able to walk short distances without assistance but neuropathic pain persisted during follow-up. VEGF and lambda light chain levels remained normal, and no monoclonal gammopathy was detectable in the serum.
Conclusion: This report of a patient with POEMS syndrome treated with ASCT suggests that severe polyneuropathy and critical overall clinical condition can improve dramatically and rather rapidly, consistent with other reports of patients with POEMS syndrome treated with ASCT. Even patients severely debilitated by POEMS syndrome appear to benefit from aggressive treatment with high-dose melphalan and autologous stem cell transplantation. These benefits need to be weighed against severe treatment related side effects.

**P921**

**An IgAL multiple myeloma showing unusual relapse patterns characterised by class switch to BJL after ABMT and development of multiple bilateral breast myelomatous lumps after reduced-intensity allogeneic bone marrow transplantation**

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Breast multiple myeloma (MM) is very rare, occurs mostly in relapsed patient lately in the course of the disease and does not have specific radiological or clinical features and can be misdiagnosed as primary breast carcinoma or as a benign process. The incidence of extramedullary MM localizations in soft tissues has increased in the last decade and a "biological" effect of new drugs as well as the immunosuppression secondary to allogeneic transplantation have been advocated as potential causes.
Herein we report an unusual case of MM who developed class switch after ABMT and multiple bilateral breast localization after reduced intensity conditioning (RIC) allogeneic bone marrow transplantation.
A 56 years old lady diagnosed IgAL MM on November 2005, she received induction therapy with thalidomide dexametasone (DEX) followed by ABMT attaining a complete remission (CR). Relapse occurred after 23 months. Noteworthy, the immunohistochemical investigation highlighted that plasmacellular infiltrate was IgA negative and expressed only L light chain. Immunofixation showed a L monoclonal component (MC) in the urine and serum while the IgA MC was no more detectable. The patients received a second lines therapy with bortezomib-DEX followed, for partial response, to therapy with lenalidomide (LEN) and DEX.
RIC bone marrow transplantation (BMT) from an HLA identical sibling was then performed on December 2009; At day 100 full chimerism and CR were documented and subsequently a limited chronic cutaneus developed. Eighteen months after RIC transplant the patients discovered a small lump (1.5 cm) in the left breast. Both mammography and echography were suspicious for malignancy and prompted a fine-needle biopsy. The histological assessment revealed a massive infiltration of L restricted IgA - IgG negative plasma cell providing the diagnosis of extramedullary relapse. In couple of weeks new breast lumps developed,L BJ proteinuria was again detected and new osteolitic lesions were documented while bone marrow showed less than 4% plasma cells and the chimerism was full donor. We started again treatment with LEN-DEX.
The breast is an unusual reported site of MM localization. Imaging studies are unspecific and small lesions can be misdiagnosed as a benign process. In our opinion in MM patients, particularly if they have received immunomodulatory drugs or allogeneic BMT, even small breast lesions should cautionary undergo histological evaluation by means of fine needle biopsy.

**P922**

**Low circulating Mmannan-binding leptin levels correlate with increased number of febrile episodes in myeloma patients who undergo high-dose melphalan with autologous haematopoietic stem cell transplantation and do not receive antibiotic prophylaxis**

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Patients with multiple myeloma (MM) are at a higher risk of infections compared to general population. Up to 80% of MM patients who undergo autologous transplantation with stem cell support (ASCT) develop neutropenic fever. The mannan binding leptin (MBL) pathway of the complement system is part of the innate immune system and seems to influence the risk of infections in cancer patients during treatment. The aim of this study was to evaluate the impact of MBL levels on the risk of infections and on the risk of development of febrile episodes in MM patients who receive HDM and ASCT. We studied 100 MM patients who underwent an ASCT between 1996-2011 in a single center. Sixty-seven patients participated in a randomized study, where in the first arm patients received prophylactic antibiotics, while in the arm B there was no antibiotic prophylaxis. In case of neutropenic fever, prophylactic antibiotics were interrupted and first line regimen was administered. In case of failure of first line treatment, a second line antibiotic combination was administered. MBL serum levels were measured in all patients on the day of mobilization, using an ELISA methodology (R&D Systems, Minneapolis, MN, USA). Seventeen (17%) patients had MBL levels <500 mg/L. Of those, 11 received antibiotics prophylaxis and 6 did not. In general, there was no statistical difference regarding the development of fever or neutropenic fever between patients with MBL serum levels of <500 mg/L or ≥500 mg/L. However, among 17 patients with MBL levels of <500 mg/L, 6 /11 patients who received antibiotics prophylaxis developed a febrile episode compared to 6/6 patients who did not receive antibiotics prophylaxis and developed a febrile episode (p=0.049). Nevertheless, patients with MBL levels <500 mg/L attained a lower response rate to first line therapy with antibiotics, i.e. lower percentage of fever resolution and higher incidence of necessitated administration of a second line antibiotic regimens, compared to patients with MBL levels of ≥500 mg/L (66.7% versus 88.9%, respectively; p=0.05). The results of our study suggests that MM patients who underwent ASCT and had low levels of MBL had a lower response rate in first line antibiotic regimens, requiring more often administration of a second more advanced line of antibiotics. Furthermore, the administration of prophylactic antibiotics to these patients seems to reduce the number of febrile episodes.

**P923**

**Weight loss of more than ten per cent and radiographic findings are the most important predictive factor for suspecting tuberculosis in patients with multiple myeloma post autologous transplant**

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Purpose: The incidence and prevalence of tuberculosis is high in India. In today's practice young multiple myeloma patients are treated with induction chemotherapy followed with high dose melphalan and maintenance chemotherapy. In our clinical practice we came across patients with multiple myeloma post transplant, are highly susceptible to tuberculosis and high degree of suspicion is required.
Aim: To determine the predictive factors to suspect the tuberculosis in patients with multiple myeloma post transplant.
Methods and Materials: We retrospectively analyzed 16 patients with multiple myeloma who underwent autologous bone marrow transplant (BMT) from January 2005 to November 2011. The entire patient fulfilled the criteria for diagnosis symptomatic myeloma. After induction chemotherapy with standard regimen patient were transplanted with 200mg/m2 melphalan as myeloablative regimen. We analyzed the predictive factors for suspecting tuberculosis in these patients.
Results: There were total of 16 patients who underwent autologous transplant, (18.7%) three patients developed tuberculosis with 6 months post BMT. We analysed for complete remission status pre trasnpalnt (p=1.15), complete remission status post transplant (p=0.56), median no of days for neutrophil to engraftment (p=0.48), fatigue (p=0.21), loss of appetite (p=0.20), loss of weight >10% of pretransplant (p=0.007), chest X ray abnormality (p=0.01).
Conclusion: Weight loss of more than 10% and chest radiological findings are most important predictive factors to diagnose tuberculosis in patients with multiple myeloma post BMT.

## Late Complications

**P924**

**Lung transplantation for Bronchiolitis obliterans syndrome after allogeneic stem cell transplantation**

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Chronic graft-versus-host-disease (cGVHD)with bronchiolitis obliterans syndrome (BOS) affecting the lungs is a serious complication after allogeneic hematopoietic stem cell transplantation (ASCT). It occurs in about 5-10% of the patients surviving > 100 days, and lung transplantation (LTx) may be the only treatment option for progressive respiratory failure. However, it is uncertain whether LTx is safe and justified in these patients, given the lack of organs and the risk of relapse of the underlying disease.
For these reasons it is common to wait for a minimum of two years after treatment of malignant disease for patients to be eligible for LTx.
The aim of this retrospective study was to evaluate this treatment based on the cases of LTx performed after ASCT in Scandinavia. All lung transplant centres in Sweden, Norway and Denmark were contacted, and data on patients with LTx after ASCT was recorded. Survival data from the Scandiatransplant register was used for comparison using log-rank test. LTx after ASCT was performed in 11 patients (<1% of all LTx in Scandinavia in the time interval). ACT was done due to chronic myeloid leukaemia (n=3), acute myeloid leukaemia (n=4), acute lymphatic leukaemia (n=2), immunodeficiency (n=1) and aplastic anaemia (n=1). All patients had clinical cGVHD, and pathological examination of the extirpated lungs showed obliterative bronchiolitis in 10 patients while one showed nonspecific fibrosis. All patients received bilateral LTx. Median age at LTx was 34 years (range 16 to 55), and the median interval from ASCT to LTx was 8 years (range 8 months to 16 years). The median observation time after LTx was 5 years (range 2 months to 15 years). Two patients have died, one due to septicaemia, the other due to relapsing leukaemia, 2 and 14 months after LTx, respectively. In these patients, the time from ASCT to LTx was 10 years and 13 months, respectively. Of the remaining, 4 patients developed BOS, of which one was retransplanted. Survival was not different in the 11 patients lung transplanted after ASCT than in patients matched for age, transplantation year and country.
Survival in our cohort is not inferior to the survival in matched controls from the Scandiatransplant register. We therefore suggest that LTx may be considered in carefully selected patients with BOS after ASCT.

**P925**

**Exercise and cardiovascular risk factors in long-term survivors of transplantation**

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Patients receiving high-dose chemoradiotherapy prior to haemopoietic stem cell transplantation (HSCT) are at increased risk of developing cardiovascular risk factors (CVRFs) compared to normal populations. CVRFs include hypertension, dyslipidaemia, and diabetes. In this study we have looked at the relationship between the average amount of exercise undertaken per week and the likelihood of developing two or more of these CVRFs in long term survivors of HSCT for haematological malignancy.
Data from routine follow up was collected on 49 consecutive patients attending a dedicated haematology-endocrine follow up clinic. The average amount of exercise done per week over the preceding year was recorded together with details of medication, blood pressure readings and laboratory data relating to lipid and glucose measurements. A patient was considered to be dyslipidaemic if they were taking a statin, if they had a cholesterol-HDL ratio above 5 or an LDL persistently above 4.
Of 49 patients, 25 were female and the median age at follow-up was 53 years (range 33-76). 41 had been treated for CML and 8 for acute leukaemia. The median follow-up time was 20 years (range 8-32) and the median dose of TBI received was 12Gy (range 10-14Gy). Patients were divided into three groups according to average weekly exercise. Group A: <2 hours (n=18), Group B: 2-4 hours (n=15) and Group C: >4 hours (n=16). In group A, 9 /18 (50%) had 2-3 CVRF; in group B, 4/15 (27%) had 2-3 CVRF and in group C, 2/16 (12.5%) had 2-3 CVRF. This trend was statistically significant (p=0.019) on a chi-squared trend test. The prevalence of 2-3 CVRF in combined groups B+C was 6/31 (19%) which was significantly lower than that for group A with a p value of 0.025 by chi-squared tests. Patient age and duration of follow up are additional variables which have been linked to the acquisition of CVRFs. In this study, there was no significant difference between the median ages of patients in groups A, B, C nor between transplant-follow up time.
In conclusion, long term survivors of transplantation undertaking more than 2 hours dedicated exercise a week on a regular basis appear less likely to accumulate CVRF.

**P926**

**Dikkopf-1 is elevated in very long-term survivors of haematopoietic stem cell transplantation**

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Skeletal abnormalities are well described following HSCT and include fractures, AVN, reduced bone density and alterations in markers of bone metabolism. We have previously described significant increases in markers of both bone formation and bone resorption in our very long term survivors of transplant. It is not clear, however, what the key influences on bone metabolism might be in ambulant very long term survivors of HSCT who are not taking steroids or calcineurin inhibitors. We have investigated this by measuring osteoprotegerin (OPG), soluble receptor activator of nuclear factor-kappa B ligand (RANKL) and dikkopf-1 (DKK-1) as important regulators of osteoclast and osteoblast function.

18 patients (9 female) were investigated a median of 16 years (range 9-29) post SCT. The median age at evaluation was 48 years (range 39-71). Patients had received cyclophosphamide (200 mg/kg) and TBI at a median dose of 13.2 Gy (range 10-14.4 Gy) as pre-transplant conditioning. All patients were euthyroid and normocalcemic and all male patients had normal testosterone levels at analysis. A pairwise comparison between patients and controls was performed using Wilcoxon signed ranks test for non-parametric samples.

Median serum levels of RANKL were 0.3 pmol/l (range 0.1-1.4) in the patient group compared to 0.08 pmol/l (range 0.001-0.96) in the controls representing a non-significant increase. Median serum levels of OPG were significantly elevated at 6.2 pmol/l (range 2.8-8.9) compared to 3.2 pmol/l (range 1.1-5-2) in the control group (p=0.001). Median serum levels of Dkk-1 were significantly increased in the patient group at 83.2 ng/ml (range 41.8-249.4) compared to 34.6 ng/ml (range 13.8-54.3) in the control group (p=0.0001). The RANKL/OPG ratio was normal.

RANKL is the major activator of osteoclast function, while OPG is the decoy receptor of RANKL and inhibits osteoclastogenesis. Dkk-1 is a member of the Dkk family of proteins which are negative regulators of the Wnt-signalling pathway and play a central role in bone formation. Dkk-1 enhances levels of RANKL and suppresses osteoblastogenesis. Our data demonstrating a significantly elevated Dkk-1 implicates alterations in Wnt -signaling in the pathogenesis of altered bone metabolism in very long-term survivors of HSCT. The rise in OPG despite elevated Dkk-1 may represent a compensatory balance effect against increased osteoclast function; the normal ratio of RANKL / OPG would support this.

**P927**

**Alterations in sex hormones after haematopoietic stem cell transplant**

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Introduction: Gonadal failure (GF) is an important risk factor for osteopenia, cardiovascular disease, sexual dysfunction, and growth delay. GF has been associated with the use of conditioning regimens (Cx) with TBI, Busulphan, and, to a lesser extent Cyclophosphamide. But the impact of other Cx on gonadal function has been less described.

Patients and Methods: We evaluated FSH (U/L), LH (U/L), estradiol (pg/mL) and testosterone (ng/mL) pre and post-HSCT (days +100 and +365) in 253 pts (140 males and 113 females) who underwent HSCT between 2008 and 2011 in our Center. Median age was 54 years (range 2-70). 109 pts had received alkylating agents (43.8%) and 39 radiotherapy (15.6%) before HSCT for the management of the disease. 37 pts had received a previous autologous HSTC. 88 (34,8%) HSCT were allogeneic (47 from family members and 41 unrelated donors). Cx consisted of: Mel 200 (76), BEAM (66), Flu-Mel (40), BEA (16), Cy-TBI (13), Flu-Bu (13), Bu-Cy (10), Flu-Cy-TBI (9), TNI (4) and others (6). 47 pts received RIC regimens (18.6%). Sources of SC were peripheral blood (n=216), bone marrow (n=29) y cord blood (n=16).

Results: Table 1 shows evolution of gonadotropins and sex hormones during de first year post-SCT according to sex and age. Among the women between 10 and 40 years old, the estradiol levels were lower than they should be for their age in 32,2% (pre-HSCT), 40% (day +100), and 54,1% (day +365), although 15,78% showed later recovery. A single case of hypogonadotrophic hypogonadism was found in a child with TBI. A 8 years old girl, without prior pubertal development, showed hormones levels consistent with menopause. Among the men aged over 50 years old testosterone levels descended in 13,8% of cases (5 over 60, 1 previous STC, 1 TBI). Only three men between 17 and 50 years had descended levels (all over 40 and with previous alkylating agents).

Conclusions: The most common hormonal alteration in our series was the hypergonadotrophic hypogonadism. A high percentage of women developed premature menopause and irreversible ovarian failure. Age was a very important factor for the development of GF in women. Few women recovered gonadal function spontaneously. Among men, the most frequent pattern was the increase in LH with normal testosterone levels (compensated hypergonadotrophic hypogonadism). In our series, melphalan conditioning was a frequent cause of gonadal failure.

**P928**

**Changes in thyroid function after haematopoietic stem cell transplantation**

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Introduction: As the survival after HSCT has improved, the identification and treatment of delayed complications has become more important. Thyroid dysfunction has been reported after HSCT, usually linked to total body irradiation (TBI) and busulphan. With the inclusion of older patients and new indications and modalities of HSCT is important to define risk factors for thyroid dysfunction.

**[P927]**

| Median | FSH (pre-HSCT) | FSH (day +100) | FSH (day+365) | LH (pre-HSCT) | LH (day+100) | LH (day +365) |
|---|---|---|---|---|---|---|
| Female <17 years | 4.20 | 36.20 | 42.25 | 1.80 | 28.20 | 17.60 |
| Female 17-40 year | 9.05 | 32.60 | 53.50 | 20.40 | 18.95 | 27.60 |
| Female >40 years | 55.70* | 66.80** | 87.50*** | 29.10* | 36.90** | 45.85*** |
| Male <17 years | 0,90 | 4.15 | 2.70 | 0.73 | 1.94 | 2.37 |
| Male >17 years | 19.95* | 22.80** | 22.00*** | 8.50* | 10.50** | 10.10*** |
| | Estradiol (pre-HSCT) | Estradiol (day +100) | Estradiol (day+365) | Testosterone (pre-HSCT) | Testosterone (day+100) | Testosterone (day +365) |
| Female <10 years | 7.45 | 4.90 | 5.10 | | | |
| Female 10-40 year | 14.50 | 5.00 | 4.90 | | | |
| Female >40 years | <4.90 | <4.90 | <4.90 | | | |
| Male <50 years | | | | 4.25 | 5.15 | 5.27 |
| Male >50 years | | | | 4.31* | 4.72 | 4.48** |

* versus **/***: p<0.05

| TSH (mU/L) | All patients (n=253) (median) | Auto HSCT | RIC | Alkylants before | Cx with Melphalan | Cx with Busulfán | Cx with ICT |
|---|---|---|---|---|---|---|---|
| Pre-HSCT | 2.17 (1.39-3.25)* | 2.22 * | 2.12 | 1.99* | 2.18* | 2.01 | 3.38 |
| Day + 100 | 2.67(1.69-3.99)** | 3.12** | 2.08 | 2.67** | 3.19** | 1.73 | 3.29 |
| Day + 365 | 2.52(1.47-4.01) | 2.62 | 2.42 | 2.60 | 2.64 | 2.09 | 3.08 |
| Thyroid dysfunction | - hypothyroidism (n=40)<br>- uncompensated (n=7)<br>- central hypothyroidism (n=9) ***<br>- decrease of TSH with normal T3 y T4 (n=3)<br>- hyperthyroidism (n=8) | | | | 19.3% | 12.5% | 41.2% |
| | *vs** p<0.05 | | ***all of them allogeneic | | | | |

Table 1. TSH at pre-HSCT, day +100 and day +365 and thyroid dysfunction

Patients and Methods: Thyroid function was prospectively tested before HSCT and at days +100 and +365 post-HSCT in 253 patients who underwent HSCT between 2008 and 2011 in our Center. 140 (55.3 %) of the patients were males. Median age was 54 years (range: 2-70). 109 patients (43.8%) had received alkylants and 39 patients (15.6%) radiotherapy before HSCT for the management of the disease. 37 patients had received previously an autologous HSTC (14.6%). Sources of hematopoietic cells were peripheral blood (n=216), bone marrow (n=29) and cord blood (n=16). Conditioning (Cx) therapy consisted of Mel 200 (76), BEAM (66), Flu-Mel (40), BEA (16), Cy-ICT (13), Flu-Bu (13), Bu-Cy (10), Flu-Cy-TBI (9), TLI (4) and others (6). 47 patients received reduced intensity conditioning (RIC) regimens (18.6%). 88 HSCT (34.8 %) were allogeneic (47 from family members, and 41 from unrelated donors).
Results: The evolution of TSH pre and post-HSCT is showed in Table 1. Compared to pre-HSCT values, TSH increased significantly in autologous HSTC, in patients previosuly treated with alkylating agents and in patients conditioned with Melphalan based regimens.
Conclusion: Hypothyroidism was frequently seen during the post-HSCT period (28,5%). Conditioning regimens with high-dose melphalan had an important effect on thyroid function. Changes in thyroid hormones were not observed in patients who received RIC regimens.

**P929**
**Vitrification of oocytes and embryos in young women after haematopoietic stem cell transplantation**
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Introduction: Conditioning regimens in HSCT are considered as a high risk factor for developing ovarian failure. Application of fertility preservation technics before HSCT in females with leukemia is controversial. The aim of this study was to evaluate the ovarian reserve in patients after HSCT with different conditioning regimens and to perform fertility preservation in young women with diminished ovarian reserve.
Patients and Methods: Ovarian reserve (AMH, AFC) was evaluated in 35 females recipients of HSCT with myeloablative (busulfan- or melphalan-based) or nonmyeloablative (cyclophosphamide-based) conditioning regimens. Mean age at HSCT was 14.5 years (range 13-17), median age at time of the study was 21 years (range 17-24). Vitrification of oocytes and embryos was performed by Cryotop method.
Results: All pts who received busulfan–based conditioning regimen (n=12) had undetectable levels of AMH and ovarian failure, whereas pts who received melphalan-based conditioning (n=8) showed diminished ovarian reserve (AMH 0.6 ± 0.2 ng/ml, AFC 4.08 ± 1.67). Ovarian reserve was normal in all (n=15) but one girl who received cyclophosphamide-based conditioning regimen; this pt developed prolonged GVHD and required long term immunosupressive therapy including steroids. Ovulation induction, OPU, vitrification of oocytes was performed in 3 patients (2 – AML, 1 – AA). Mean number of retrieved/vitrified oocytes was 5/4. Vitrification of embryos (n=3) was performed in 1 patient with AML.
Conclusion: Vitrification of oocytes or embryos is feasible in young women after HSCT and can be regarded as a preferred fertility preservation method. Early planning of fertility preservation gives a chance to realize reproductive function in future in patients with diminished ovarian reserve.

**P930**
**The successful pregnancy in patient following allogeneic transplant with busulphan-based conditioning regimen for AML**
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We report a rare case of a 23 year-old female who had ovarian function recovery and a normal full term delivery after hormone-replacement therapy (HRT) through 9 years after allogeneic HSCT with busulphan-based conditioning regimen for AML.
The conditioning regimen consisted of busulfan 16 mg/kg, cyclophosphamide 120 mg/kg, and Liposomal daunorubicin 200 mg/m$^2$. Successful engraftment of donor cells and full donor's chimerism was achieved without signs of leukemia.
One year after the HSCT the patient received a course of HRT as a treatment of hypergonadotropic hypogonadism and absence of menses. After 12 months of the HRT recovery of ovarian function was confirmed. Eight years after the HSCT spontaneous pregnancy occurred; heartbeat of the fetus was registered on week 7. Three weeks later a non-severe vaginal bleeding occurred and ultrasound examination showed a

non-developing pregnancy. Genetic examination of the abortion material revealed a full triploid genotype (69 XXX).
After 4 months spontaneous pregnancy occured again. At 20 weeks of gestation woman was advised by geneticist. According to the results of studies of genetic markers, the genetic risk of fetal chromosomal pathology is regarding as low. All clinical and laboratory parameters were within normal limits. The whole period of pregnancy was uneventful. At 37-38 weeks' gestation the patient was admitted to the hospital with preterm premature rupture of membranes. Considering this, as well as signs of chronic fetal hypoxia, Cesarean section was performed. A girl infant was delivered with a birth weight of 3492g and Apgar scores of 4 and 7 at 1 and 5min, respectively. Postpartum period was unremarkable. On the 6th day the mother and newborn were discharge in satisfactory condition.
To our knowledge this is a first case of ovarian function restoration and spontaneous pregnancy in a AML patient after multiple courses of high dose chemotherapy and allogeneic transplant with busulphan-based myeloablative conditioning.

**P931**

**Incidence and predicting factors of abnormal thyroid function test in adult patients post haematopoietic stem cell transplantation at King Hussein cancer centre**

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Introduction: The risk of abnormal thyroid function after hematopoietic stem cell transplantation (HSCT) is well-known especially in pediatric patients. There are fewer studies about adult patients that showed 3-year cumulative incidence ranges between 8-23%. In our study we studied the incidence of abnormal thyroid function in patients older than 18 years, and reviewed the predicting factors for it.
Patients and Method: This is a retrospective study for all adult patients who underwent HSCT at KHCC stem cell transplant program from 2007-2011.
138 file were reviewed, 30 of it were excluded because the patients had abnormal thyroid function before transplant, so our cohort consisted of 108 patients who had normal thyroid function before transplant.
In our cohort 66% were males. Lymphoma & myeloma constituted 57.4%, Acute leukemia accounted for 24%, aplastic anemia for 5.6%. From the group 13.9% had more than one transplant, 54.8% had autologus transplant, 45.2% had allogenic transplant from which 94.6% had myeloablatibe transplant. The conditioning regimen was TBI-based in 29%.
Results: With median follow up of 1.1 year, 34 patients (31%) developed abnormal thyroid function tests, high TSH accounted for 40.7%, low TSH for 38.9%, low T4 for 11.1%, with high TSH and low T4 for 3.7%.
The predicting factors which showed significant p value were: Gender: the incidence in the female group was 42.9%, compared to 24.2% in the male group (p 0.042) Type of transplant: the incidence in the allogenic group was 40.5% compared to 19.6% in the autologus group (p 0.027) TBI-based conditioning versus non-TBI: the incidence in the TBI group was 44.4% compared to 22.7% in non-TBI group (p 0.036). The disease category, number of transplants, and myeloablative versus nonmyeloablative were not predictors of the occurrence of abnormal thyroid function test. From the 34 patients who developed abnormal test after transplant, 61.7% of them developed this in the first year post transplant, and 20.5% in the second year.
Conclusion: The incidence of abnormal thyroid function after HSCT in our study is around 30%. The most common abnormalities are high or low TSH. Most of the abnormalities happened in the first 2 years after transplant.
Female gender, allogenic transplant, and TBI-based conditioning were associated with more occurrences of thyroid function abnormalities.

**P932**

**Marked improvement in osteoporosis and bone pain with double-biphosphonate therapy in patients with Thalassaemia major undergoing allogeneic stem cell transplantation**

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Bone mineral density (BMD) reduction is a known complication in thalassemia major and Allogenic Haemopoietic Stem Cell Transplantation (Allo-HSCT). Several factors are implicated in bone mass reduction in thalassemia such us delayed sexual maturation, hypothyroidism, ineffective haemopoiesis with progressive marrow expansion. Therefore, these patients present osteoporosis or osteopenia at HSCT. In controlled studies calcium Vit D3 and bisphophonates (BI) have been used to prevent and cure severe bone loss following HSCT. To evaluate the safety and efficacy of BI to cure bone loss and acute bone pain after HSCT,14 thalassemic major patients (7 M/7 F;age 5-25 yrs),who had undergone HSCT from matched unrelated and sibling donors between 2007-2010, were enrolled. BMD was measured by dual X-ray absorptiometry (DEXA) scan at baseline, 12, 24 and 36 months following HSCT,while bone metabolism by urinary C-telopeptide (C-T) of collagen type I and serum Osteocalcin (OC, alkaline phosfatase (bALP) levels, were evaluated at DEXA. Six-twelve months after transplant,four patients reported severe back and leg pain with motor difficulties. In these patients, calcium and D3 vit was initiated at 6 months after transplant and sex hormone replacement therapy as soon as was possible. Therapy with cloridronate i.e. at 300 mg 3 times a week for 2 weeks and once a week for 4-5 weeks was also started. As soon as a decrease in pain was observed, intravenous neridronate therapy, 25 mg once a month for 2-3 yrs was initiated. The remaining 10 patients received 25 mg of neridronate i.e once a month for 2-3 yrs. Notable response to cloridronate and neridronate treatment was seen in all 4 patients with severe bone pain. A marked BMD increase was also observed in all 14 patients in the L1-L4 and femoral neck regions. The T-score range for adult patients and the Z-score range for child patients prior to osteoporosis treatment was > -2.5-5.5 DS and following therapy -1.5 - 0.5 DS, P value 0.004. No statistical differences prior to or after BI treatment in OC, bALP, C-T levels; P value 0,11 were observed .Conclusions: Our data demonstrate that BMD reduction in the L1-L4 and femoral neck regions is common in thalassemic transplantation patients. Cloridronate was found be safe and effective in the management of bone pain resulting from osteoporosis and Neridronate was highly effective in increasing BMD and decreasing severe osteoporosis in the post transplantation period.

**P933**

**Bone status and endocrine dysfunctions in adult long-term survivors 10–15 years after allogeneic stem cell transplantation**

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Bone loss and endocrine dysfunctions are potential late complications of allogeneic stem cell transplantation (allo-SCT). Long-term data on these issues after allo-SCT in adults are limited.
Objectives: A prospective study on bone status and endocrine functions was performed in 20 survivors who underwent allo-SCT for leukemia as adults at Karolinska University Hospital for 10–15 years ago.
Methods: Patient characteristics are presented in Table 1. Bone mineral density (BMD) was assessed by a total-body dual-energy X-ray absorptiometry (DEXA). Blood samples were collected for analysis of: osteocalcin, PTH, 25-hydroxy-vitamin D, calcium, phosphate, bone specific ALP, TSH, free T4, testosteron, SHBG, FSH, LH, estradiol, and IGF-1. Urine samples were collected for analysis of NTx/creatinine. A study questionnaire aimed to identify factors influencing analyzed variables.

[P933]

Table 1. Patient characteristics

| Variable | Results |
|---|---|
| Number of patients (F/M) | 20 (8/12) |
| Age mean (range) | 49 (34–69) |
| Weight (kg) mean (range) | 74 (52–104) |
| Height (cm) mean (range) | 174 (153–191) |
| BMI (kg/m²) mean (range) | 24 (17–35) |
| CML/AML | 11/9 |
| Age at transplantation mean (range) | 36 (22–58) |
| Matched related/unrelated donor | 10/10 |
| Myeloablative/Reduced-intensity conditioning | 19/1 |
| TBI based conditioning | 14 |
| Chronic GVHD | 13 |
| Time from BMT (years) mean (range) | 13.4 (11–15) |
| Duration of corticosteroid therapy (months), mean (range) | 35 (0–162) |
| Duration of immunosuppressive therapy (months), mean (range) | 39 (0–162) |
| Smokers yes/no | 3/17 |
| Hormone replacement therapy F/M | 7/1 |
| Bisphosphonate therapy at study time F/M | 2/0 |

Results: A low BMD for age was found in one woman at the total hip and in one man at the femoral neck. One patient has lost >3 cm in length; one man experienced an elbow fracture; one man and one woman got unilateral aseptic necrosis of the femoral head. Four patients with the lowest Z-score at the total hip had also the lowest Z-score at the femoral neck.
Values of studied hormones are showed in Table 2. An elevated level of plasma PTH was disclosed in 6 of 20 (30%) patients. One patient had hypercalcemia due to parathyroid adenoma; in 5 patients there was no clear explanation for the PTH elevation. A low value of 25-hydroxy-vitamin D (<45 nmol/L) was seen in 9 of 20 (45%) patients. Three of 6 patients with elevated PTH concentrations had low vitamin D values.
All women received estrogen replacement therapy after allo-SCT until expected age of menopause. One man received testosterone; 4 other men had testosterone below the lower normal limit. Elevated FSH values were present in 8 men. All but one patient showed normal values of the thyroid tests. Three patients had IGF-1 values below the lower limit of the reference range.
Conclusions: Adult long-term survivors of allo-SCT have relatively well preserved BMD 10–15 years after allo-SCT. Prophylactic treatment of osteopenia should be individualized, but control of BMD is necessary as a part of follow-up. Low levels of vitamin D and hyperparathyroidism were common. Control of PTH and vitamin D levels before and after allo-SCT is recommended and vitamin D supplementation should be considered if indicated.

**P934**

**Effect of dysimmunity on blood group determination after allogeneic stem cell transplantation: a difficult case**

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Aim: Description of blood determination problems through one case of an allogeneic hematopoietic stem cell transplantat (HSCT) patient presenting high level of auto-antibodies.
Description: A 13 year-old boy, known for a previous severe fulminant hepatitis that was followed by very severe aplastic anaemia (VSAA) was treated by antithymocyte globulin and ciclosporine, without response. Unrelated fully matched HSCT after a conditioning regimen consisting of anti-thymocyte globulin (ATG, Fresenius®), fludarabine and cyclophosphamide, using the Geneva protocol of T-cell depletion by Campath® followed by T-cell add back was performed. Major blood group incompatibility (patient A Rh positive, donor O positive) represented a risk of haemolytic anaemia. Engraftment was uneventful and cutaneous GvHD grade I developed one month later. Chimerism was of 100% donor origin. The patient developed a mild renal thrombotic microangiopathy associated with renal failure and severe haemolytic anaemia that was treated with corticosteroids. Persisting A/O Rh positive blood group was observed using monoclonal gel method (Diaclon ABO/D Bio-Rad) due to A and O blood transfusion after allo-HSCT. At 22

[P933]

**Table 2. Endocrine status of long-term survivors 10–15 years after allo-SCT**

| Biochemical measurement (range) | mean (SD) |
|---|---|
| Plasma creatinine µmol/L (<100) | 83.8 (26.6) |
| Plasma calcium mmol/L (2.15–2.5) | 2.33 (0.12) |
| Plasma albumine g/L (36–45) | 38.45 (3.84) |
| Serum ionized calcium mmol/L (1.15–1.33) | 1.27 (0.07) |
| Plasma PTH ng/L (10–65) | 64.45 (37.41) |
| Plasma phopsphate mmol/L (0.75–1.4) | 0.99 (0.19) |
| Plasma magnesium mmol/L (0.7–0.95) | 0.66 (0.06) |
| Serum 25 hydroxy-vitamin D nmol/L (75–250) | 54.85 (26.8) |
| Serum TSH mE/L (0.4–3.5) | 2.07 (0.92) |
| Serum free T4 pmol/L (8–14) | 10.15 (1.26) |
| Serum FSH E/L; M/F (1–12.5) | 16.26 (9.29) / 59.87 (30.9) |
| Serum LH E/L; M/F (1.2–9.6) | 7.79 (3.27) / 42.9 (30.9) |
| Serum estradiol pmol/L – females | 39.88 (22.74) |
| Serum testosteron nmol/L – males (10–30) | 11.69 (3.29) |
| Serum SHBG nmol/L; M/F (20–70) | 39.16 (9.94) / 37.25 (26.5) |
| Serum IGF-1 mikrog/L (85–220) | 121.6 (35.72) |
| Serum ALP bone specific µg/L (<20) | 12.94 (3.12) |
| Serum osteocalcin µg/L (<50) | 19.4 (7.83) |
| Urinary NTx/creatinine nmol BCE/mmol (3–51); n=16 | 37.31 (13.26) |

months post allo-HSCT, blood group determination still showed a double blood group population in the absence of transfusions during more than 3 months. Direct Coombs test was strongly positive for IgG and C3d. The presence of a high titre of non-specific autoantibody was observed. There was no hematologic sign of relapse but severe haemolytic anaemia was unresponsive to steroid treatment. Blood group determination was repeated after washing the red blood cells with NaCl 0.9% and proceeding with enzymatic digestion with papaine that result in the donor's O Rh positive blood group.
Conclusion: Blood group determination is an important analysis in the follow up of patients after HSCT, predicting relapse and, in case of differences of blood groups between donor and recipient, haemolytic anaemia. Our case shows technical difficulties of blood group determination of unknown reaction in a dysimmune status probably due to presence of high titer of auto-(or allo-) antibodies. Transplant physicians should be aware of such complications and be in closed contact with the immuno-hematology laboratory to be able to determine the real group and adapt treatment strategy.

**P935**

**The prevalence of impaired glucose tolerance and metabolic syndrome in long-term survivors of haematopoietic stem transplantation recipients: the effect of plasma adipohormone, endothelin-1, insulin like growth factor- 1, tumour necrosis factor-alpha leve**

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In recent years clinical and laboratory findings of metabolic syndrome (MS) with an incidence of 25-49% have been reported in adult and pediatric long term survivors of HSCT. The aim of our study was to determine the prevalence of impaired glucose tolerance (IGT) and MS in long term survivors of HSCT.A total of 83 HSCT recipients (39 autologous, 44 allogeneic; median age 33 years, range 16-64) who survived ≥ 1 year after HSCT were included in the study. Patients were median 3 years (range 1-7 years) status post HSCT. Nineteen of forty four patients (43.8%) of allogeneic HSCT recipients had chronic GVHD during enrollement. The conditioning regimen included total body irradiation in 11 out of 44 (13.3%) recipients while it was non- myeloablative in 13 out of 44 (29.54%) allogeneic HSCT recipients. MS was diagnosed

according to the National Cholesterol Education Program-Adult Treatment Panel III (NCEP- ATPIII). MS was diagnosed in 14 patients (16.86%). IGT was detected in 9/39 (23.07%) of autologous and 6/44 (13.63%) of allogeneic HSCT recipients. IGT was more frequent in patients with MS compared to ones without MS (5 out of 15 patients with MS *vs* 9 out of 68 patients without MS) (p=0.06). Median age of patients with MS was 43 years (range 31-60) while it was 28 years (range 16-64) in patients without MS (p=0.002). Four of the 14 patients with MS and 10 out of 69 patients without MS had active underlying disease (p=0.241) during the time of evaluation. Plasma levels of TNF-alpha, ESR, CRP in patients with and without MS did not show a significant difference (p>0.05). Plasma adiponectin levels were significantly higher in patients with MS (p=0.019). However plasma leptin levels were significantly lower in patients with MS (p=0.013). Plasma endothelin–1, IGF–1, and iron parameters were similar in patient with and without MS (p>0.05). Median plasma leptin and adiponectin levels were significantly higher in women than men (p<0.05).
Conclusion: Prevalence of MS in adult Turkish population was reported as 26-28%. The frequency of MS is relatively lower than the previous reports which might be due to the shorter follow- up time. Chronic GVHD and immunosuppressive treatment was present in a significant proportion of our allogeneic HSCT recipients including the 2 patients with MS. Altered adipocytokine levels and IGT seems to play role in the development of MS. Our results should be validated with a larger sample size and a longer follow-up.

**P936**

**Oral cavity chronic-graft-versus host disease after allogeneic haematopoietic stem cell transplantation**

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The oral cavity is one of the most affected organs of cGvHD. In this retrospective study, we aimed to present our experience on the incidence and risk factors oral cavity cGvHD.
Patients and Methods: Total 503 patients underwent allo-HCT between Jan 2000 and Dec 2010. We observed cGvHD in 226 patients (55.8%) surviving more than 100 days after the transplantation (n=405). Oral cavity cGVHD was seen in 74 out (32.7%) of the patients with cGVHD. But we retrospectively evaluated only 64 patients (31M; 33F) developing oral cavity cGvHD with acceptable data. Median age was 38 years (range: 14-57 ys). Using the NIH assessment criteria oral chronic GVHD was scored as mild, moderate and severe.
Results: The distributions of severity of oral cavity cGVHD were 28.1% mild (n=18), 42.2% moderate (n=27) and 29.7% (n=19) severe as the scoring system at median 6.2 months from the transplantation. In two patients oral cavity GvHD developed as a part of acute GvHD and progressed to cGVHD. Isolated oral cavity cGVHD was seen in only two patients. Oral cavity GvHD developed in 20.3% of the patients prior to systemic extensive GvHD. In our cohort analysis, 53.1% of the patients had a history of smoking prior to transplantation. In univariate analysis we did not observe any pre- and postransplantation risk factors on severity of oral cavity GvHD (Table). Most of the patients required systemic immunosuppressive treatment with topical agents. We observed that oral cavity cGVHD improved in only three patients with only topical treatment.
Conclusion: Isolated oral cavity involvement may predict the development of extensive GvHD. Therefore, we should determine in which patients require systemic immunosuppressive treatment or not. In addition, large-well design comparative studies needed to evaluate the risk factors of the development and severity of oral cavity cGVHD.

**Table:** Pre- or posttransplant-related factors and severity of Oral Cavitiy GvHD

| Grade of oral cavity GvHD | Mild (%) | Moderate (%) | Severe (%) | p |
|---|---|---|---|---|
| Gender (Male) | 38.8 | 55.5 | 47.3 | 0.5 |
| Smoking (Present) | 50.0 | 66.6 | 10.9 | 0.1 |
| Stem cell source (Peripheral Blood) | 88.8 | 88.8 | 94.7 | 0.8 |
| Total body irradiation | 11.1 | 25.9 | 31.6 | 0.3 |
| Intensity of conditioning regimen (Ablative) | 27.8 | 18.5 | 26.3 | 0.8 |
| Methotrexate | 83.3 | 85.2 | 68.4 | 0.3 |
| Acute GvHD | 50 | 40.7 | 47.4 | 0.8 |

**P937**

**Efficacy and safety of deferasirox after allogeneic stem cell transplantation: results of CICL670AES04 trial**

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Objectives: The aim of this trial was to determine the efficacy and safety of deferasirox in pts with iron overload after allogeneic stem cell transplantation (SCT).
Methods: Phase IV, open-label, multicenter trial. Main eligibility criteria: age ≥18 years old, SCT at least 6 months before inclusion, transfusional iron overload (serum ferritin (SF) ≥1000 ng/mL or >20 units red blood cell transfusions), serum creatinine (SCr) ≤2-fold the upper limit of normal (ULN) or creatinine clearance ≥50 mL/min. Treatment regimen consisted of 10 mg/Kg/day of deferasirox 52 weeks or until SF≤400 ng/mL; subsequent adjustments were based on SF levels and safety markers. Primary objective was to assess the reduction of SF after 52 weeks of treatment.
Results: From December 2008 to April 2010, 30 pts were enrolled in 18 centers. Median age was 46 years (range 20-65). Underlying primary diseases included acute myeloid leukemia (n=17), myelodysplastic syndrome (n=6) and non-Hodgkin's lymphoma (n=5). Pts had received the SCT a median of 12.3 months before deferasirox initiation (range 5.9-39.2). At week 52 there was a significant reduction in median SF from baseline (by LOCF: -670 ng/mL; p<0.05). Median SF (range) at baseline was 1444 ng/mL (788-4055; n=30), and at week 52 was 756 ng/mL (96-7326; n=30 LOCF). Liver iron concentration (LIC) was assessed by MRI (gradient echo sequences). At week 52 there was a significant reduction in median LIC (-8.9 mg Fe/g dry weight (dw); p<0.05). Median LIC at baseline was 13.4 mg/g (range 5.6-19.0; n=12) and after 52 weeks was 4.6 mg/g (range 0.0-12.3; n=8). Eight pts completed the study after achieving SF≤400 ng/mL before week 52. Eight pts discontinued the study prematurely due to: disease progression (n=3), death (n=2), consent withdrawal, SCr increase (<2xULN) and unsatisfactory therapeutic effect. Most common investigator-assessed drug-related AEs were gastrointestinal events (n=4 pts,1 3.3%), SCr increase (<2xULN) (n=4 pts,13.3%) and increased transaminases (n=2 pts, 6.7%). Nine Serious AEs (SAEs) were reported in 8 pts: 3 primary disease progressions, 1 febrile neutropenia, 1 herpes zoster, 1 fever with rhinorrhea, 1 leukocytosis, 1 massive acute subdural hematoma and 1 febrile syndrome; none of them considered to be related to deferasirox.
Conclusion: Deferasirox provided significant reduction in SF levels and LIC over 1-yr treatment in patients post allogeneic SCT, with a safety profile similar to that reported in previous studies.

**P938**

**Non-invasive evaluation of liver iron overload and stiffness in patients undergoing allogeneic stem cell transplantation**

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Aim of the Study: We evaluated liver iron overload (LIO) in 77 patients who underwent an allogeneic hemopoietic stem cell transplant (HSCT), because of haematologic malignancies.
Patients: The patients were considered eligible for iron overload evaluation, if they had a positive history of transfusional and a serum ferritin (SF) level > 500 ng/ml.
Iron overload was assessed by Biosuscettometry Magnetic Iron Detector (MID) and expressed as grams of iron in the whole liver: our current cut-offs are as follows; <1gr no LIO detectable, 1-3 gr mild LIO; >3 g high LIO. Stiffness was assessed by Fibroscan® and converted in Metavir hepatic fibrosis score.
Results: 25 patients had undetecable LIO with median SF 1413 ng/ml (507-5000), median stiffness 6.3 KPa (3.7-11) equal to metavir F0-F1. 30 patients (38%) and had mild LIO with median SF 2015 ng/ml (576-4288) and median stiffness 5.85KPa (3.8-9) equal to metavir F0-1. 22 patients (28%) had high LIO with median SF 4311 ng/ml (537-5000) equal to metavir F0-F1and median stiffness 9.5 KPa (3.8-14) equal to metavir F2. Patients with undectable LIO had a transfusional intake <25 RBCU (8-24), patients with mild and high LIO had a transfusional intake >25 RBCU (25-85).
We then stratified patients for time from SCT in 3 groups: less than 6 months, 6-214 months, over 2 years. The Stiffness and LIO were similar in the 3 groups; there was a modest decrease in ferritin levels, from a median of 3513 (<6 months) to 2000 (>2 years), In conclusion liver iron overload is present in a high percentage of patients (66% – moderate or severe-) following SCT. Iron overload does not seem to decrease with time. This result calls for appropriate chelating treatment post-SCT.

**P939**

**Efficacy and safety of therapeutic phlebotomy for iron overload in allogeneic haematopoietic stem cell transplantation recipients**

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Iron overload may occur in up to 60% of allogeneic hematopoietic stem cell transplant recipients (alloHSCT) and is associated with a poor outcome. Few studies have analyzed iron depletion strategies in alloHSCT patients.
This retrospective study aimed to assess the feasibility and efficacy of therapeutic phlebotomy in allo-HSCT recipients. Chart review was performed for all patients from our centre who were included in a phlebotomy program between january 2005 and january 2011.
During the study period, 23 allo-HSCT patients (78 % male) were treated by regular phlebotomy. Initial disease was acute leukemia in 16 patients (70%). Ten patients (44%) were transplanted with an HLA-identical sibling donor. Conditioning regimen was myeloablative in 14 (61%) patients. The median age at time of phlebotomy initiation was 46 (26-67) years and the median time elapsed from transplantation to the first phlebotomy was 7 (1.6-196) months. Notably, 11 patients (47 %) started therapy within the first 6 months post-transplantation. All patients were transfusion independent at time of therapy initiation.

**[P939]**

| | Before phlebotomy therapy initation | After completion of phlebotomy therapy | Wilcoxon signed ranks two-tailed test p-value |
|---|---|---|---|
| **Iron metabolism** | | | |
| Serum ferritin (µg/l), median (range) | 1949.5 (720-5536.17) | 467 (89-2051) | P< 0.001 |
| **Liver function tests** | | | |
| AST (times upper level), median (range) | 28.63 (17-93) | 23.4 (9-47) | P< 0.04 |
| ALT (times upper level), median (range) | 42.13 (18-197.17) | 27.5 (9.5-62.5) | P< 0.001 |
| GGT, median (range) | 56.34 (25.5-466.4) | 45.5 (7.67-122) | P = 0.006 |
| PAL, median (range) | 94.92 (41.5-134.33) | 56 (34.33-115) | P <0.001 |
| Bilirubin, median (range) | 16 (8.5-35) | 12.25 (5-19) | P = 0.001 |
| LDH, median (range) | 233.34 (139-324) | 183 (118.5-464.5) | P = 0.005 |
| Albumine, median (range) | 37.25 (32-43) | 39 (33.5-48) | P = 0.02 |

and 11 patients (47%) received in addition an erythrocyte-stimulating agent (ESA) to facilitate phlebotomy. At time of data cut-off, the median follow-up was 46 (24-217) months. The median duration of the phlebotomy program was 24 months (range, 1.6-60) and the median number of procedures required to decrease ferritin below the target level of 500 ug/l was 16 (range, 4-56). In total, a median of 4.8 L (range, 0.9-23.8) of whole blood was removed from each patient. The median ferritin level was 1949 (range, 720-5536) ug/l before phlebotomy initiation compared to 467 (89-2051) ug/l after completion of the program (p<0.001). Interestingly, patients who completed the program exhibited significantly improved liver function tests, as shown in Table 1. Overall, phlebotomies were well tolerated and no adverse events were recorded during or after blood collection.

Our study shows that phlebotomy is a safe and effective method for reducing iron overload in allo-HSCT recipients, and in that respect, it should be considered the treatment of choice because of its low-cost. In patients with persistent anemia, notably in the early post-transplant period when the risk of iron-overload related complications is relatively high, the use of an ESA should be considered since it may substantially facilitate phlebotomy.

**P940**

**Gastro-esophageal reflux disease and its association with Bronchiolitis obliterans syndrome in haematopoietic stem cell transplant recipients**

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Introduction: Bronchiolitis Obliterans Syndrome (BOS) is a significant and serious late complication in HSCT recipients. It affects 6-10% of post-HSCT patients with overall survival of only 13% at 5 years. The etiology of BOS is poorly understood and is believed to be heterogeneous with multiple factors influencing its pathogenesis and progression. Most commonly accepted view is an immunological theory which involves lymphocyte mediated inflammatory and fibrotic process that affects the epithelium and sub-epithelium of the airways, leading to narrowing and airflow limitation. Recent data is emerging to reflect an association of BOS with gastro esophageal reflux disease (GERD) mainly in post lung transplant patient. There are no studies on post-HSCT patients with BOS and its association with GERD.

Methods: This is a prospective observational study in which seven patients with established diagnosis of post-HSCT BOS, based on classical pulmonary function test (PFT) findings consistent with obstructive airway disease and CT chest finding according to the current diagnostic criteria were evaluated for presence of GERD by the Bravo catheter-free radio pH capsule.

Total acid exposure time, using pH values of 4.0 or below in the distal esophagus, was used to quantify reflux. A DeMeester score (normal value < 14.72) was also calculated for each patient for day 1, day 2.

Results: Six out of seven patients completed BRAVO pH study for 48 hours while one patient recorded only for 12 hours due to technical failure. Five patients, four males and one female, with a mean age of 34.2 years were reported to have positive acid reflux study. Three patients had severe while two had mild reflux.

Transient dysphagia and/or chest discomfort were reported by 3 patients.

Conclusion: The above study suggests that GERD is a possible contributing factor for BOS and/or its progression in post HSCT patients. Larger studies are needed to confirm these findings. Our study may help in future management of BOS patients post allogeneic HSCT with aggressive anti GERD therapy including gastric fundoplication as a beneficial response of aggressive anti GERD therapy has been documented in post lung transplant patients with BOS.

**P941**

**Risk factors and outcome of late-onset non-infectious pulmonary complications after reduced-intensity allogeneic transplants**

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Late-onset non infectious pulmonary complications (LONIPCs) were recognized as cause of morbidity and mortality after myeloablative HSCT; however, incidence, risk factors and outcome of this complication are less studied after reduced intensity conditioning (RIC) HSCT. We prospective followed 177 consecutive adult patients with hematologic malignancies who underwent RIC HSCT between April 1999 and December 2009 with pulmonary function tests (PFTs) before transplant and at 3, 6 and 12 months and, when clinically required, with high resolution thorax computed thomography and bronchoscopy.

**[P940]**

**Table 1: Bravo Capsule pH Monitoring Results**

| No. of patients | Gender | Age | Yrs since HSCT | GVHD | Fraction time pH <4 (%) on Day 1 | Fraction time pH <4 (%) on Day 2 | Total fraction time pH <4 (%) in 48 hours | Total DeMeester Score (Day 1 & Day 2) |
|---|---|---|---|---|---|---|---|---|
| 1 | F | 44 | 10 | Y | 2.5 | 6.8 | 4.6 | 16.3 |
| 2 | M | 27 | 3 | Y | 3.8 | 5.2 | 4.5 | 17.9 |
| 3 | M | 47 | 7 | Y | 14.9 | 9.8 | 12.4 | 37.5 |
| 4 | M | 25 | 4 | Y | 9.0 | - | - | 35.2 |
| 5 | M | 28 | 3 | Y | 17.3 | 17.5 | 17.4 | 59.7 |
| 6 | F | 35 | 2 | Y | 3.5 | 1.8 | 2.7 | 10.8 |
| 7 | M | 28 | 3 | Y | 0.7 | 1.1 | 0.9 | 3.0 |

Of the 124 patients surviving at least 3 months, 11 (8, 9%) developed LONIPCs at a median time of 14 months (5-43) after HSCT and were further subclassified as having bronchiolitis obliterans (6 patients), bronchiolitis obliterans with organizing pneumonia (2 patients), and interstitial pneumonia (3 patients). Overall, 4/11 patients obtained a partial response after steroids alone or combined with cyclosporine or mycophenolate mofetil. The remaining 7 patients were unresponsive to salvage treatments; six of them died at a median of 6,5 months after the diagnosis of LONIPCs due to respiratory failure or infections. Two-year TRM cumulative incidence was significantly higher in LONIPC than in non-LONIPC patients (64% *vs.* 23%, p=0.006). However, since 2-year relapse incidence was significantly lower in LONIPC than in non-LONIPC patients (0% *vs.* 43%, p=0.002), we couldn't observe any significant difference in OS. Univariate analysis showed that GvHD prophylaxis with ATG is a protective factor (0.17, CI 0.03-0.85, p=0.03), while moderate to severe chronic GvHD is a strong significant risk factor for LONIPCs (R 16.9, CI 2.08-136.77, p=0.008) and is the only significative factor in multivariate analysis. PFTs before transplant did not differ between the 2 groups. However, FEV1 and FVC values significantly differed since the 3th month after HSCT and through the first year of transplant between LONIPCs and non-LONIPCs patients (p<0.007 and p<0.008, respectively). DLCO values had a trend to become lower in LONIPCs patients in comparison with unaffected ones only at 12 months after HSCT (p=0.06).
We conclude that the development of LONIPCs after RIC HSCT significantly increased TRM and was strongly associated with moderate and severe chronic GVHD. This complication could be prevented by the administration of ATG before HSCT and by periodical PFTs through the first year after transplant.

**P942**

**Histopathological analysis of chronic kidney disease post allo SCT**

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Background: Chronic kidney disease (CKD) is one of the common complications following allogenic hematopoietic stem cell transplantation (allo SCT). However, there have been few detailed clinicopathological investigations of CKD after allo SCT.
Purpose: To investigate the clinicopathological features of treatment-related injury to renal tissue, and to investigate glomerular changes associated with immunological abnormality.
Methods: We performed clinicopathological analysis of 10 patients who had undergone renal biopsy in our institution between 2003 and 2010 for CKD post allo SCT.
Results: Median age at SCT was 36.5. Conditioning regimen was myeloablative for 7 cases and non-myeloablative for 3 cases. GVHD prophylaxis was FK/CyA base for 6/4 cases, respectively. Renal biopsies were conducted at a median of 591 days (range 226 to 4995 days) after allo SCT. Indications for renal biopsy included decreased eGFR (7 cases), proteinuria and edema (7), or both (4). The treatment-related renal tissue damage, mainly ischemic changes were seen in most of the cases. The median period of calcineurin inhibitor administration was 711.5 days (56-4995). There were correlations between the total period of calcineurin inhibitor administration and the calcineurin inhibitor toxicity score (CNIT score) consisting of pathological tubular and interstitial changes and glomerular changes. Five of 10 cases had glomerular findings associated with immunological abnormalities (3 membranous nephritis, 1 focal segmental glomerulonephritis, and 1 minimal change nephrotic syndrome). They received immunosuppressive therapy and two had developed brochiolitis obliterance. Clinical nephrotic syndrome had occurred not only in these five cases showing specific changes in glomeruli, but also in other cases which had severe renal tissue injury.
Conclusion: The total period of calcineurin inhibitor administration correlated with the severity of pathological renal impairment. Glomerular diseases associated with immunological abnormalities occurred post allo SCT, and responded well to immunosuppressive therapy. In nephrotic syndrome, renal biopsy may be useful to determine whether to use immunosuppressive therapy.

**P943**

**Hyperbaric oxygen therapy for severe late onset haemorrhagic cystitis in children who underwent haematopoietic stem cell transplantation**

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Objectives: Hemoragic Cystitis (HC) is a common cause of morbidity and extended hospitalization after hematopoietic stem cell transplantation (HSCT). Many therapies have been investigated to prevent or treat HC (mesna, hyperhydratation, ciprofloxacin, antiviral agents, prostaglandin, etc.) based on multifactorial ethio-pathogenesis, but effective treatment is still indeterminate. While the efficacy of hyperbaric oxygen therapy (HOT) has been established for HC due to chemotherapy and/or radiation therapy, its role in HC occurring after transplant has yet to be defined. We report our single center experience with HOT in late onset grade II-III HC after HSCT.
Methods: HC was defined as the presence of persistent gross hematuria and symptoms of bladder irritability without urinary tract infection. A preventive treatment with prehydration and mesna has been administered in patients (pts) receiving cyclophosphamide. HOT has been associated with a concomitant treatment with oral oxybutynin, hyperhydratation and/or irrigation of the bladder through catheter. Cidofovir has been administered based on demonstration of viral infection.
Treatment with HOT consisted of breathing 100%O2 for 75 minutes total in the hyperbaric chamber (3 cycles of 25 min each with 3 min break) to the absolute pressure of 2.5 atmospheres with a minimum of eight sessions. The resolution of the HC was defined as hematuria disappearance within 7 days from the stop therapy.
Results: During the period 2005-2011, 186 HSCT have been performed c/o our institution in 158 pediatric pts. Ten pts out of 186 (6%) developed grade II-III HC. Clinical and transplant features have been summarized in Table 1.
HC developed in the median day+26 (range:+20;+47) after the HSCT. HOT was started in the median day+31 (range: +20;+56). After a median of 10 sessions (range:8-30) 7/10 pts (70%) achieved a complete remission (CR). The procedure was well tolerated. No pts died for HC. Two children did not benefit from HOT: one needed a cistostomy and the other interrupted HOT after 26 sessions because of no response.
Conclusions: HOT is a well tolerated procedure also in the paediatric setting. To date, there are no evidence-based guidelines for the treatment of HC. Our data show that the precocious beginning of HOT might be effective in the treatment of HC offering advantages in terms of prognosis, potential sequelae and resolution of hospitalization. Each patient needed of a variable number of sessions to reduce symptoms.

| CLINICAL CHARACTERISTICS | | HSCT | | | | | | | | | | HEMORRAGIC CYSTITIS | | | | | Outcome Post-BMT (months) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | FEATURES | | | | | ENGRAFMENT | | COMPLICATIONS | | | | | | | | |
| Patient Sex / Age (years) | Diagnosis | Donor Type | Stem cell source | Conditioning regimen | GVHD Prophylaxis | TNC/ Kg | PMN (day) | PTL (day) | Acute GVHD | CMV ReAct | BK Inf. | HC Onset (days post-BMT) | Start HBO (days post-BMT) | HBO Sessions (No.) | HC Resolution (day) | Outcome HC | |
| M/10 | AML | Auto+ Purging | BM | Bu,Cy,L-PAM | CSA ,MTX | 3,94 x 10*8 | 23 | 26 | | no | ? | 20 | 26 | 10 | 39 | well | + |
| M/ 8 | AML | MUD | BM | Bu,Cy,L-PAM | CSA | 3,12 x 10*8 | 18 | 20 | no | no | ? | 27 | 28 | 8 | 43 | well | ANED |
| F/10 | ALL | MSD | BM | Bu,TT,Cy | CSA ,MTX | 1,4 x 10*8 | 18 | 26 | no | no | ? | 47 | 48 | 15 | 61 | well | ANED |
| M/8 | ALL | MUD | BM | Bu,TT,Cy, | CSA,MTX, ATG | 2,56 x 10*8 | 16 | 25 | no | yes | ? | 16 | 32 | 30 | 69 | Cystotomy | + |
| M/6 | AML | MUD | BM | Bu,Cy,L-PAM | CSA,MTX, ATG | 5 x 10*8 | 16 | 18 | no | yes | ? | 3 | 20 | 10 | 34 | well | ANED |
| M/6 | AML | MUD | PBSC | Bu,Cy,L-PAM | CSA,MTX, ATG | 1,5 x 10*7 | 11 | 14 | II-III | no | ? | 24 | 26 | 11 | 50 | well | + |
| M/6 | ALL | MUD | BM | TBI,TT,Cy, | CSA, ATG | 3,77 x 10*8 | 15 | 25 | II | yes | ? | 14 | 46 | 10 | 56 | well | ANED |
| M/8 | ALL | MSD | BM | TBI,THIO,Cy | CSA,m-PDN | 2,58 x 10*8 | 15 | 2 | II | yes | no | 30 | 37 | 12 | 52 | well | ANED |
| F/ 7 | MDS (KD) | MUD | Cord | Bu,Cy,L-PAM | CSA,m-PDN, ATG | 5,7x 10*7 | 21 | 46 | no | yes | yes | 28 | 31 | 8 | 53 | No compliance, SR | ANED |
| M/ 7 | ALL | MUD | Cord | Bu,Cy,L-PAM | CSA ,MTX, ATG | 10.5x 10*7 | 26 | no* | no | yes | yes | 41 | 56 | 26 | 93 | SR | ANED |

**Table 1.**
The features of the patients are summarized in the table.
Abbreviations: AML= acute myeloid leukemia; ALL= acute lymphoblastic leukemia; MDS= myelodysplastic syndrome; KD= kostmann disease; Auto= autologus transplantation; MUD= matched unrelated donor; MSD= matched sibling donor; BM= bone marrow; PSBC= peripheral stem blood cells; BU= busulphan; L-PAM= melphalan; THIO= Thiotepa TBI= total body irradiation; CSA= cyclosporin; m-PDN= methilprednisolone; MTX= metothrexate; ATG= anti-thymocyte globulin; TNC= total nucleated cells; CMV ReAct= CMV reactivation; Bk inf.= BK virus infection; HC= hemorragic cystitis; HBO= hyperbaric oxygen; SR= spontaneous resolution; ANED= alive with non evidence of disease; †= death

**P944**
**Structural changes of myocardium and state of RAAS in children and adolescence after allogeneic haematopoietic stem cell transplantation**
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Background: Allogeniec haematopoietic stem cell transplantation (alloHSCT) is the treatment of choice in patients (pts) with different hematological and inherited diseases. Increasing numbers of alloHSCT are being performed annually with a greater number of long-term survivors. There is increasing concern regarding the late complications and long-term effects that are secondary to performed therapy. Recipients experience mortality rates higher than the general population and the risk of premature cardiovascular death is increased 2.3-fold compared with the general population. One of leading cause is development metabolic syndrome with activation renin-angiotensin-aldosterone system (RAAS).
The Aim of this Study: To study cardiac function in pts after alloHSCT, investigate RAAS as a marker of myocardial fibrosis.
Patients: After alloHSCT 20 pts were under investigation (12–boys, 8–girls) with follow-up 4-12 year (median - 7,8+ 2.2 y). At the moment of alloHSCT age of patients was 2-15.6 yo (median 8.4+3.2). Type of diagnosis: ALL-11 (55%), AML-5 (25%), CML-3 (15%), MDS -1 (5%) pts. Routinely ECG, echocardiography, the level of aldosterone and rennin were performed.
Results: All pts did not have any signs of heart failure and any structural changes in myocardium: ejection fraction (EF) was 50-75% (median 61.5+7.6%). But boundary values EF (50-53%) were revealed in 2 pts (10%), 8 pts (40%) had hypertension in pulmonary artery. The level of renin and aldosterone were higher in transplanted pts than in the control group: 1.07 *vs* 0.32 ng/ml*h, 106.0 *vs* 41.2 pg/ml*h, respectively. These changes were correlated with period of follow-up after alloHSCT. In pts with long-term follow-up hormone levels was higher than the others.
Conclusion: There were not signs of cardiac dysfunction by clinical date, ECG, echocardiography up to 8 years after alloHSCT. But activation of RAAS was found as possible marker of initial structural damage of heard. Monitoring will determine participation of RAAS in pathogenesis of myocardium damage after alloHSCT and develop protocols for support of cardiac dysfunction in initial subclinical stage.

**P945**
**Chronic graft-versus-host disease is a risk factor for hyposplenism after allogeneic stem cell transplantation**
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Objectives: Howell Jolly bodies (HJB) are nuclear fragments within the red blood cells that are normally removed by the spleen. The appearance of HJB in the peripheral blood smear is the most characteristic abnormality after splenectomy or in patients with functional asplenia and can be used as surrogate marker of hyposplenism. We observed in a number of patients following allogeneic hematopoetic stem cell transplantation (HSCT) HJB in the blood smear. We hypothesize, that patients with chronic Graft versus Host disease (cGvHD) have a high prevalence of functional hyposplenism, and are therefore at risk for severe infections.
Methods: We performed a cross-sectional analysis of all outpatients seen between 1.7.2009 and 30.6.2011 following autologous or allogeneic HSCT at our Transplantation Center. Patients with surgical splenectomy were excluded. The total number of patients included into the study was 511. There were 389 (76%) patients treated with allogeneic, and 122 (24%) with autologous HSCT, with a median follow up after HSCT of 72 months (range, 1-406). Patients with HJB after allogeneic HSZT were compared with those without HJB, using a univariate and multivariate analysis (logistical regression). The patients' characteristics are shown on Table 1.
Results: 63/511 patients presented HJB in their blood smear during the study period. The probability of finding HJB was significantly higher ($p<0.0001$) in patients following allogeneic HSCT (61;97%) than in patients following autologous HSCT (2;3%). Due to the low number of patients with HJB after autologous HSCT, the subsequent analysis was resricted to patients after allogeneic HSCT only. 87% of the patients with HJB had cGvHD compared to 59% of the patients without HJB ($p<0.0001$). Furthermore, patients conditioned with TBI, those who received peripheral blood stem cells (instead of BM), and male patients had significantly more often HJB (Table 2). In the multivariate analysis, the presence of cGVHD (RR, 7.04; 95%CI

2.409-20.365; p<0.0001), TBI (RR, 2.185; 95%CI, 1.130-4.224; p=0.020) and male recipients (RR2.517;95%CI 1.223-5.181; p=0.012) were associated with a higher probability of HJB.
Conclusion: Chronic GvHD is the most important independent risk factor for the development of hyposplenism after allogeneic HSCT and is only rarely observed after autologous HSCT. The clinical relevance of these findings and the consequences in the management of transplanted patients will be discussed.

**[P945]**

Table 1: **Patients' characteristics**

| | |
|---|---|
| Total number of patients evaluated in outpatient clinic | 536 |
| Patients without splenectomy (***Patients included into the study***) | **511** |
| Type of HSCT | |
| - Allogeneic HSCT | 389 (76%) |
| - Autologous HSCT | 122 (24%) |
| Sex oft he patients | |
| - Males | 308 (60%) |
| - females | 203 (40%) |
| Median age at HSCT, years (range) | 46 (6-73) |
| Median age at last control, years (range) | 50 ( 11 -76) |
| Median Follow-up, months (range) | 72 (1-406) |
| Source of hematopoietic stem cells | |
| - Bone marrow | 84 (16%) |
| - PBSC | 422 (83%) |
| - Cord blood | 5 (1%) |
| Diagnosis | |
| - Acute leukemia | 170 (33%) |
| - CML | 53 (10%) |
| - MDS/MPN | 58 (11%) |
| - Myeloma | 95 (19%) |
| - CLL, NHL, HD | 100 (20%) |
| - SAA | 18 (4%) |
| - others | 17 (3.3%) |
| Risk category | |
| - Standard risk | 407 (80%) |
| - High risk | 104 (20%) |
| In allogeneic HSCT | |
| Myeloablative Conditioning | 253 (79%) |
| Conditioning with TBI (>7.5 Gy) | 198 (51%) |

**P946**
**A case of posterior reversible encephalophathy syndrome triggered by sun and heat exposure in a patient who underwent allogeneic bone marrow transplantation and with a history of cyclosporine neurotoxicity**
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Introduction: The Posterior Reversible Encephalopathy Syndrome (PRES) has previously been described in patients

**Table 2: Comparison patients with and without Howell Jolly Bodies (Univariate analysis)**

| *parameter* | *Howell Jolley bodies* | | *P-value* |
|---|---|---|---|
| | *With* | *without* | |
| Type of HSCT | | | |
| - Allogeneic HSCT | 61 (97%) | 328 (73%) | |
| - Autologous HSCT | 2 (3%) | 120 (27%) | <0.0001 |
| Patients with allogeneic HSCT | | | |
| Sex of the patients | | | |
| - Males | 43 (70%) | 193 (59%) | 0.087 |
| - females | 19 (30%) | 135 (41%) | |
| Median age of the patients at HSCT | 44 (19-68) | 43 (6-70) | 0.395 |
| Age groups at HSCT | | | |
| - ≤ 45 years | 35 (57%) | 179 (55%) | 0.686 |
| - > 45 years | 26 (43%) | 149 (45%) | |
| Age at last FU | 51 (24-69) | 50 (19-72) | 0.502 |
| Time interval (months) | 59 (1-360) | 60 (1-406) | 0.785 |
| Source of HSC | | | |
| - Bone marrow | 5 (8%) | 76 (24%) | |
| - PBSC | 55 (91%) | 246 (75%) | 0.008 |
| - Cord blood | 1 (1%) | 4 (1%) | |
| Diagnosis | | | |
| - Acute leukemia | 30 (49%) | 127 (39%) | |
| - CML | 8 (13%) | 43 (13%) | |
| - MDS/MPN | 7 (11%) | 50 (15%) | 0.319 |
| - Myeloma | 5 (8%) | 31 (9%) | |
| - CLL, NHL, HD | 11 (18%) | 51 (16%) | |
| - SAA | 0 | 17 (5%) | |
| - others | 0 | 9 (3%) | |
| Risk category | | | |
| - Standard risk | 44 (72%) | 260 (79%) | 0.215 |
| - High risk | 17 (28%) | 68 (21%) | |
| Myeloablative conditioning | 44 (80%) | 209 (79%) | 0.813 |
| TBI (>7.5 Gy) | 39 (64%) | 159 (49%) | 0.028 |
| Acute GVHD | | | |
| - No GVHD | 21 (37%) | 105 (34%) | 0.652 |
| - With GVHD | 36 (63%) | 206 (85%) | |
| Chronic GVHD | | | |
| - None | 8 (13%) | 134 (41%) | < 0.0001 |
| - With chronic GVHD | 53 (87%) | 194 (59%) | |

receiving immunosuppressive therapy for the prophylaxis of Graft-Versus-Host Disease (GVHD). It has been suggested that cyclosporine (CSA) could damage the blood–brain barrier through direct toxic effects on the vascular endothelium, vasoconstriction caused by elaboration of endothelin, and microthrombosis. The heat stress or dehydration-induced hyperthermia results in significant reduction in blood flow to the brain. In rats the prolonged exposure to heat determined an increase in the concentration of vasopressin.

Patient: One female patient (pt) of 27-year-old with Hodgkin's lymphoma had undergone allogeneic bone marrow transplantation (ABMT) from haploidentical mother in third complete remission. The conditioning regimen included Thiotepa 5 mg/Kg day -6, once-daily i.v. Busulfan 3,2 mg/Kg/daily and fludarabina

50 mg/m²/daily from day -5 to -3 and ATG Fresenius 5 mg/Kg/daily from day -5 to -2. The combination of CSA, mycophenolate mofetil, methotrexate and basiliximab has been used for prophylaxis of Graft Versus Host Disease. On day +25 the pt showed a diffuse skin rash treated with steroid therapy. CSA was the only immunosuppressive drug after the first 100 days from ABMT and was tapered to suspension for neurological toxicity (tremor). For the persistence of the tremor during the week of suspension, the pt underwent magnetic resonance imaging (MRI) of brain and lumbar puncture, which were negative. Cerebrospinal fluid was analyzed and the PCR for BKV, JCV, CMV, EBV, HSV 1-2 was negative. For appearance of skin rash, the CSA was restored (50 mg/daily) in association with steroid therapy. On day +264, the pt exposed herself to sun and heat (outside temperature of 29 °C) showing of headache, mental confusion, aphasia, generalized seizures and only an episode of high temperature (39°C). The EEG showed diffuse slow activity (theta-delta) mainly in the frontal lobe. The MRI of the brain showed bilateral perivascular white matter low density lesions in parieto-occipital lobes. It was the diagnosis of PRES. We withdrew CSA and the pt showed the disappearance of neurological symptoms within 36 hours. MRI control performed after one month was completely negative.
Conclusion: We suppose in this case that the PRES has been triggered by the combination of effects on cerebral vessels by CSA, by the exposure to heat and by the consequent hyperthermia.

**P947**
**Transverse myelitis caused by Varicella-Zoster virus infection following haematopioetic stem cell transplantation in a patient with Thalassaemia major**
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Occurrence of transverse myelitis during or following varicella infection is uncommon. No diagnostic test is completely accurate, since varicella zoster (VZV) can not usually be isolated from blood or cerebrospinal fluid (CSF) in VZV myelitis. We describe a case of transverse myelitis in a patient with thalassemia major, during a proven VZV infection following hematopioetic stem cell transplantation (HSCT). A 16-year-old girl with thalassemia major underwent HSCT from her full matched brother. Full engraftment was achieved after partial splenic arterial embolization and second transplantion because of the first graft failure. Acyclovir prophylaxis were given in the early post-transplantation period. She was transfusion-independent with full single-donor chimerism and with good health condition after 7 months of the second transplant, until she had presented in February 2011 with low back pain, paresthesia and weakness in both legs, a painful itchy vesicular eruption in her lumbosacral area and urinary incontinence. Neurologic examination revealed normal levels of cooperation, orientation and intact cranial nerves. The patient exhibited 2/5 lower extremity weakness on the right and 3/5 on the left, absent abdominal reflexes below the level of T2 dermatom. Her gait was markedly ataxic. Deep tendon reflexes were normal in the upper extremities, but decreased in the lower extremities. Babinski sign was bilateral positive. CSF was positive for VZV PCR. Spinal cord MRI at the time showed intramedullary lesions of high signal intensity on T2-weighted sequences between the T2 and L1 levels, findings which are consistent with myelitis. The patient was diagnosed with transverse myelitis, but despite the treatment with acyclovir, methylprednisolone, intravenous immunoglobulin, hyperbaric oxygen and physical therapy there is no improvement in her situation. There is no consensus on the dose, duration and type of the antiviral prophylaxis after HSCT, but it is known that antiviral prophylaxis is effective at delaying the onset of VZV after HSCT, but not affect the overall incidence of infection. Further studies are needed to show whether more prolonged prophylaxis, given until effective cellular immunity is re-established, can further reduce or even totally abrogate excess susceptibility to VZV reactivation.

**P948**
**Possible role of apelin in development of metabolic syndrome in children treated with stem cell transplantation**
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Background: Metabolic syndrome (MS) incidence in patients treated with haematopoietic stem cell transplantation (HSCT) is higher than in general population and similar to that found in obese people. The most important consequence of MS is low-level systemic inflammation supported by adipose-specific synthesis of proinflammatory soluble molecules. Hormones from adipose tissue are also strongly involved in MS development. The purpose of the study was to determine concentration of chosen peptides secreted from adipose tissue in children treated with HSCT.
Patients and Methods: Nine patients (pts.) 3,3-17,8 (average 10,6) years old, 6 boys and 3 girls, diagnosed with AA-3, ALL-4, CGD-2 treated with HSCT according to EBMT protocols were included to the study. The control group was composed of 9 children 3,6-14,6 (average 10,7) years old, 6 boys and 3 girls with obesity. The blood was collected in fasting state, and at 60 and 120 minutes during oral glucose tolerance test. The HSCT children were studied before and after HSCT (mean 9 months), the control group was tested once. There were no signs and symptoms of the primary disease in children after HSCT, all of them completed immunosuppressive treatment. Plasma levels of resistin, apelin, visfatin, adiponectin, leptin and leptin soluble receptor were measured using commercially available EIA kits.
Results: BMI before HSCT was 12,8-27,4 (average 19,7), BMI Z-score -2,88-2,05 (average 0,1). BMI after HSCT was 13,9-28 (average 20,4), BMI Z-score -1,2-2,01 (average 0,35). BMI in the obesity group was 21,8-36,4 (average 29), BMI Z-score 1,57-3,9 (average 2,3). The only significant difference was increase in mean apelin concentrations in fasting state and 60 min after glucose intake comparing to mean concentrations before HSCT. The same relationship were noticed compering obesity group and children before HSCT in third time point (120 min. after glucose intake).
Conclusion: As apelin is strongly involved in development of MS due to its role in decreasing insulin secretion, downregulation of catecholamine-mediated lipolysis and insulin resistance, it is possible that increased concentrations of apelin observed in children after HSCT may be a reason of higher incidence of MS in patients treated with HSCT.
Study sponsored by national grant number K/PBW/000520 and University grant number K/ZDS/000984.

**P949**
**Late relapse in a long-term survivors with AML after autologous transplantation in first remission - presentation of two cases**
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Introduction: After attainment of an initial remission, patients with AML can receive consolidation therapy with either chemotherapy or hematopoietic-cell transplantation (HCT) based on various prognostic factors (age, performance status and cytogenetic). Autologous HCT has also been investigated as consolidation therapy for AML and can extend survival in a select subgroup of patients.
Aim: To present long-term survival in patients with AML in first CR treated with autologous transplant and the possibility of late relapse.

Case Report: We present two cases with AML (AML FAB 2 and AML FAB5a), males 52 and 47 years on diagnosis, treated with autologous transplantation in first CR during 1999 and 2004. Remission induction was achieved by 2 cycles of chemotherapy with ADE, followed by consolidation treatment with HDAC. Stem cell mobilization was performed by chemotherapy and G-CSF, including VP-16 2 g/m$^2$ and G-CSF in the first patient and HDAC and G-CSF in the second which resulted in poor mobilization and insufficient graft. BM was chosen as source instead. Both cases received Bu-Cy conditioning and PBSC as source in one patient 2,8x10$^6$/kg CD34+ cells and the other patient received cryopreserved BM with 3,2x10$^8$/kg mononuclear cells. Engraftment was registered after day +12 in the PBSC patient and on day +17 in BM patient. Both patients were followed by an outpatient basis during 3 years after transplant. Disease relapse was registered after 12 years in the first case and after 7 years in the second case. Flowcytometry revealed no change in the leukemia initial immunophenotype which ruled out the possibility for “de novo” AML.
Conclusion: The mortality rates of AML patients who receive an autologous HCT and stay in remission for 2-years are similar to that of the general population. However, late effects of transplantation such as second cancers and other organ specific late complications can take many years to develop and studies that include an adequate number of very long-term survivors are still needed to realize the complete risks and impact of late mortality following autologous HCT for AML. Also the possibility of late relapse of the same malignancy should not be underestimated which opens the questions of how long do we need to follow up transplant survivors.

**P950**

**Incidence, treatment options and outcome of isolated extramedullary relapses after allogeneic haematopoietic stem cell transplantation fo acute leukaemias: an updated single-centre experience with 459 patients**

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Introduction: Isolated extramedullary relapses (IEMR) are increasingly reported as long-term complications after allogeneic hematopoietic stem cell transplantation (alloHSCT) for acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). However, the optimal treatment strategies for IEMR following alloHSCT remain uncertain.
Aims and Methods: We retrospectively analysed incidence, clinical features, treatment options and long-term outcome of this pattern of leukemia recurrence in a group of 459 consecutive patients (pts) (169 with ALL, 290 with AML) who underwent alloHSCT in our institution between June 1993 and June 2010. 78 pts (36 with ALL, 42 with AML) relapsed (any site). RESULTS. 12 (15%) out of all pts who relapsed (4 with high-risk B-line ALL, 1 with T-line ALL, 7 with AML, F/M 7/5, median age 35 years, range 28 – 54 years) developed histologically proven IEMR after a median time of 14 months (mts) (range, 6 – 80 mts) following alloHSCT. 5 pts (3 with ALL, 2 with AML) developed skin and/or subcutaneous tissue infiltrates. Other sites of IEMR included (No. of cases/diagnosis): leptomeninges of the brain (1/ Ph+ ALL), paraspinal soft tissues (1/AML), small intestine (1/AML), lymph nodes (1/AML), paranasal sinuses (1/AML), pleura (1/ALL), breast (1/AML). Treatment plans for those IEMR included (No. of cases/diagnosis): 1/involved-field radiotherapy (IF-RT) followed by chemotherapy (CHT) and interferon-alpha (2/ ALL), 2/ imatinib + CHT + steroids and methotrexate intrathecally (1/ ALL), 3/ imatinib + CHT (1/ ALL), 4/ CHT (2/ AML, 2/ALL), 5/ dasatinib (1/ CD117+ AML), 6/surgery (1/AML), 7/ CHT and secondary alloHSCT (2/AML). 8/12 pts died after a median time of 13,5 mts (range, 1 – 30 mts) due to resistant systemic relapse and/or infectious complications, 4/12 pts are currently under CHT or after secondary alloHSCT.
Conclusions: Our data indicate that IEMR following alloHSCT are a common occurrence in pts with acute leukemias. Sites of relapses vary widely among the pts, however, in most of them leukemic infiltrates are localized outside the well-defined sanctuaries (central nervous system or testis), predominantly within the skin and/or subcutaneous tissue. IF-RT seems to be effective initial treatment option, but it does not prevent from systemic relapse and should be followed by other therapeutic modalities. Due to the lack of efficacious treatment strategies, there is a need for novel approaches to manage IEMR after alloHSCT.

**P951**

**Malignancies after allogeneic haematopoietic stem cell transplantation – a single-centre analysis of 370 patients**

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A risk of secondary (sec) malignancies increases after allogeneic hematopoietic cell transplantation (HCT). We analyzed posttransplant malignancies in 370 allogeneic HCT recipients (335 adults/35 children) transplanted at Turku University Hospital in years 1981 to 2011. We found 16 (4.8%) adult patients with 19 sec malignancies and one child with lymphoma. Median age at transplant was 38 (4-66) years. The indications for HCT were: acute myeloid leukemia 7, chronic leukemia 3, aplastic anemia 2, lymphoma 2, multiple myeloma 2, myelodysblastic syndrome (MDS) 1.
Secondary malignancies (n=20): The areas of sec malignancies were: head-neck-mouth 7 patients (35% of malignancies), gastrointestinal tract 5 (25%), lungs 3 (15%), and one of each in mammae, prostata, gynaecological, MDS and carsinoma (ca) in situ abdominis. 35% of all were squamocellular-cas. One adult had four different malignancies post HCT. The median time from transplant to diagnosis of malignancy was 6.8 (0.6-18.2) years. Ten patients (63%) died of the sec malignancy with a median of 1.5 (week-10.3 years) years from the diagnosis. The sec malignancy was the primary cause of death in 6% of all posttransplant deaths.
A sec malignancy occurs most often after one year from HCT. In our cohort there were 221 patients (60%) who had survived longer than one year from HCT. Of them, 78% had received myeloablative (MA) conditioning and 22% reduced-intensity conditioning (RIC), and in these groups 12 (7%) and 2 (4%) patients, respectively, had developed a sec malignancy. With regard to occurrence of chronic graft versus host disease (cGVHD), 19% of patients had extensive cGVHD and 81% had it not, and the respective figures for sec malignancies were 7 (16%) and 7 (3.9%), respectively.
In conclusion, we found 17 patients (5%) out of 370 transplanted patients with a sec malignancy, and 14 patients (6%) in patients surviving more than one year posttransplant. A sec malignancy was the primary cause of death in 6% of all post-transplant mortality. The risk of sec malignancy was 8.8 times higher than that in the general Finnish population. The size of our transplant cohort is relatively small but it seems that there is a relationship between extensive cGVHD and malignancy. On the contrary, the impact of myeloablative conditioning *vs* RIC on occurrence of sec malignancy was not evident.

**P952**

**Secondary neoplasms and mortality analysis after autologous stem cell transplantation: a 10-year single-centre experience**

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Introduction: For autologous stem cell transplantation (ASCT) early transplant related mortality (TRM) is very low (1-2%) and the main cause of failure is relapse.

Recently a published series diffuse large Bcell lymphoma (DLCBL) has alerted about a high NRM over time after ASCT; the development of secondary solid tumors,myelodysplasia and acute myeloid leukemia is also a matter of concern in the long term.
We reviewed our series of ASCT patients retrospectively to assess late toxicity and efficacy.
Patients and Methods: From November 2001 to November 2011, 77 ASCT were performed to 73 patients for hematologic malignancies, 50 male and 27 female of median age 48.5 years (23-69).
Primary diagnoses included 33 multiple myeloma (MM), 21 non hodgkin lymphoma(9 DLCBL,8 follicular lymphoma (FL), 1 anaplastic lymphoma and 3 peripheral T cell lymphoma), 17 Hodgkin lymphoma (HL), 4 acute leukemia (3 myeloid,1 lymphoblastic) and 2 amyloidosis. 41.5% patients received a transplantation in complete remission, 50.6% in partial remission and 7.8% were refractory. 61% of them in first line and the rest as salvage therapy.
PBSC (peripheral blood stem cell) mobilization was performed with G-CSF in 41 and G-CSF + chemotherapy in 36 cases. The conditioning regimen was melphalan 200 for MM, escalated CBV and BEAM for lymphoma, BEA for AML and BUCY2 for ALL.Ten patients received radiotherapy before and 5 after ASCT. Median number of chemotherapy regimens before transplant was 1 (1-5).
Results: Median time from diagnosis to transplant was 8.5 (5-145) months (m.Early TRM was 1.3%. With a median follow up of 62.5 m (0.25-121) 72.8% of patients are alive and 54.5% without progression. Late mortality was 25.9% and the main cause of death was relapse (95%); NRM was 1.3%: one patient suffered a sudden death of cardiac origin. Median time to death was 20 months (2-117). Two patients (2.6%) has developed secondary solid tumors: 1 colon cancer and 1 testicular cancer. One patient has a myelodysplastic syndrome (1.3%). The 5 years OS (overall survival) and PFS (progression free survival) for MM was 72.7% and 56.5%, for DLBCL 66.7% and 77.8%, for FL 62.5% and 50% and for HL 64.7% and 35.3%.
Conclusion: ASCT is a safe procedure in our experience, with a low early TRM (1.3%). Relapse was the main cause of mortality (24.7%). NRM was also low (1.3%). We have not found a high incidence of secondary MDS/AML (1.3%) or solid tumors (2.6%), may be because of a relatively short follow-up and a cohort of patients not heavily pretreated.

**P953**

## Clinical characteristics and outcome of patients relapsing 2 years or more following allogeneic stem cell transplantation for chronic myeloid leukaemia

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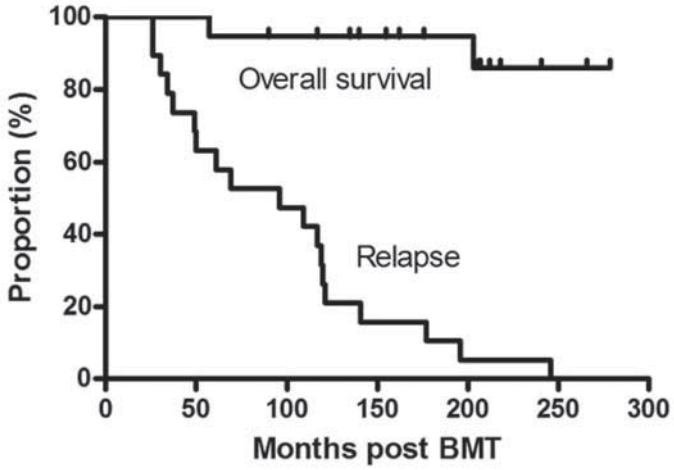
Introduction: Prior to the widespread availability of the tyrosine kinase inhibitor imatinib mesylate (IM) for the treatment of chronic myeloid leukaemia (CML), this disease was the commonest indication for allogeneic stem cell transplantation (BMT). The majority of patients (pts) treated with BMT were apparently cured. The observation that some relapses occurred late after BMT prompted a review of these pts to identify their clinical characteristics and outcomes.
Patients and Methods: 157 consecutive pts undergoing BMT for CML between 1986 and 2002 were evaluated and eligible pts were those who survived at least 2 years and then relapsed with CML. Patient characteristics and post-transplant events were identified from the databases of the units and detailed chart reviews. No pts were excluded from the analyses. At the time of transplant, 15 pts were in first chronic phase, and 4 were in accelerated phase, with a median age of 40 (range 19-55 years). Conditioning regimens were BuCy (n=13), CyTBI (n=4), FluMel (n=1) and BuMel (n=1). Donor types included sibling (n=17), matched unrelated (n=1) and one antigen mismatched family member (n=1), with stem cell sources of bone marrow (n=18) and peripheral blood (n=1). Graft versus host disease prophylaxis for all pts was with cyclosporin and methotrexate .
Results: Eligible pts were identified of whom 19 relapsed with CML more than 2 years post transplant. Median time to relapse was 96 months (range 26-246 months). The majority of pts relapsed with molecular or cytogenetic markers only (n=17), with 2 having overt haematologic disease. All pts received treatment post relapse. Donor lymphocyte infusion (DLI) (n=7), IM (n=9) or both DLI and IM (n=1) were given to the non-haematologic relapses who were treated with a second BMT or chemotherapy. 17 pts achieved a molecular remission and are alive in ongoing molecular remission, while 2 pts died from disease progression (see Figure). Of 10 pts treated with IM, 8 have ceased the drug and remain in molecular complete remission (CR) more than 2 years post cessation.
Conclusion: While BMT is curative for the majority of pts with CML in chronic phase, a proportion will relapse, sometimes up to 20 years post BMT. Ongoing vigilance with molecular

**[P953]**

**Time to relapse and overall survival of 19 patients with CML relapsing > 2 years post allogeneic BMT**



monitoring for BCR-ABL is required as intervention with IM or DLI or both usually results in rapid molecular CR and long term disease free survival. Cessation of IM is possible in a significant number of these patients achieving molecular CR.

**P954**

**Comparative evaluation of the predictive power of the EBMT risk score and the Hematopoietic Cell Transplantation Co-morbidity Index for allogeneic haematopoietic stem cell transplantation in a treosulfan-based conditioning setting**

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Background: In the attempt to clarify if allogeneic hematopoietic stem cell transplantation (allo-HSCT) provides a better outcome than non-transplant strategy in high-risk hematological malignancies, several algorithms were developed to assess the pre-transplant risk. Optimize the pre-transplant risk assessment is a crucial point to improve the allo-HSCT decision making process. To date 2 major algorithms are of use in the clinical practice: the EBMT risk score and the Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) risk score.
We are here reporting the results of the evaluation of both the EBMT and the HCT-CI risk-assessment in our patient population to guide decisions making process in a patient oriented transplant strategy.
Methods: Risk assessment score and outcome analysis included consecutive patients receiving transplant at our Institution. Data were obtained from local database.
Results: Between Jan-2004 and Nov-2011, 427 pts received an allo-HSCT in the fulfillment of clinical indications of EBMT recommendations. Data for a complete evaluation of both EBMT and HCT-CI were available for 247 allo-HSCT (pts and disease characteristics are listed in Table 1).
The median HCT-CI score was 2 and the EBMT risk score 4. In our patient population we observed a correlation between lower EBMT risk score and lower HCT-CI risk score (chi-squared test, p0.058 - Table 2).
We were not able to demonstrate an impact on OS dependent on HCT-CI score, while the impact was demonstrated according to EBMT risk score.
The overall survival (OS) at 26 months was 52% (±4%). Among 107 pts dead, 47/107 died due to disease relapse, 60/107 due to transplant related mortality (TRM).
The 26 moths OS according to EBMT risk score stratification was: 83% score 0-2, 56% score 3-4, 36% score 5-7 (p <0.001); 68% score <4 versus 42% score ≥4 (p <0.001). The evaluation of the HCT-CI impact after EBMT risk score stratification was not able to show a significant difference in outcome, despite the correlation between lower EBMT risk score / lower HCT-CI.
Conclusions: We are aware of some major limitation of our analysis - such as the sample size, the heterogeneity of the disease under treatment, the short follow-up - but it is of interest to observe how, in this setting, HCT-CI was not able to predict outcome while EBMT risk score did. Further evaluation, in a larger and more homogeneous setting, will be recommended to better evaluate the correlation between this two risk score algorithms.

**[P954]**

**Table 1** – Patients characteristics

| | 247 HSCT |
|---|---|
| **Age**, years | |
| Median | 48 |
| Range | 17-76 |
| **Follow-up**, months | |
| Median | 26 |
| Range | 0-84 |
| **Conditioning regimens**, "n" and % | |
| Treosulfan based | 231 - 94% |
| Others | 16 - 6% |
| **Disease diagnosis**, "n" and % | |
| Acute Myeloid Leukemia | 131 (53%) |
| Acute Lymphoblastic Leukemia | 31 (13%) |
| Myeldysplastic syndrome | 23 (9%) |
| Non Hodgkin Lymphoma | 19 (8%) |
| Hodgkin Disease | 12 (4%) |
| Others | 31 (13%) |
| **Disease status at transplant**, "n" and % | |
| Complete Remission (CR) / early | 131– 53% |
| Progression of Disease (PD) | 105 – 43% |
| Very Good Partial Remission (VGPR) | 11 – 04% |
| **Donor Type**, "n" and % | |
| MRD | 55 – 22% |
| MUD | 72 – 29% |
| UCB | 08 – 03% |
| Haplo-SCT | 112 – 46% |
| **Number of this transplant**, "n" and % | |
| 1 | 215 – 87% |
| 2 | 27 – 11% |
| 3 | 05– 02% |
| **HCT-CI score distribution**, "n" and % | |
| 0 | 52 – 21% |
| 1-2 | 99 – 40% |
| 3-4 | 74 – 30% |
| >/=5 | 22 – 9% |
| **EBMT risk score distribution**, "n" and % | |
| 1 | 08 – 03% |
| 2 | 30 – 13% |
| 3 | 60 – 24% |
| 4 | 48 – 19% |
| 5 | 61 – 25% |
| 6-7 | 40 – 16% |

**P955**

**Analysis of patients undergoing autologous haematopoietic stem cell transplant in our hospital**

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Objectives: We analyze the evolution of patient after autologous hematopoietic stem cell trasplant and the importance of pre-transplant factors with the frequency of relapse and survival time.
Material and Methods: We study 48 patients diagnosed between January 2007 and December 2010: median age 51 years (19-67), 33 men (68.8%) and 15 women ( 31.3%). The diagnoses

**[P954]**

**Table 2** – EBMT score and HCT-CI score correlation analysis (chi-square test)

| | | EBMT risk score | |
|---|---|---|---|
| | | <4 | >/=4 |
| HCT-CI | <2 | 46 – 47% | 52 – 35% |
| | >/=2 | 52 – 53 % | 97 – 65% |

p0,058

| | | RELAPSE (21 patients) | DEATH (14 patients) |
|---|---|---|---|
| Age | > 40 years old | 61,9 % | 78,5 % |
| | < 40 years old | 38,1 % | 21,4 % |
| Diagnostic | GM | 52,3 % | 42,8 % |
| | LNH | 14,2 % | 21,4 % |
| | EH | 9,5 % | 0 % |
| | LA | 19 % | 28,5 % |
| | OTHERS | 4,7 % | 7,1 % |
| RTpre | YES | 14,2 % | 14,2 % |
| | NO | 85,7 % | 85,7 % |
| RTcond | YES | 23,9 % | 21,4 % |
| | NO | 76,1 % | 78,5 % |

were: Non-Hodgkin›s Lymphoma (NHL) in 8 patients (41.7%), monoclonal gammapathy (MG) in 14 (29.2%), Hodgkin disease (HD) in 8 (16.7%), leukemia Acute in 5 (10.4%) and prolymphocytic leukemia in 1 patient (2.1%). Establish the incidence and timing of relapse, overall survival and mortality unrelated to the transplant. We analyze the impact of age, diagnosis and the radiation therapy prior to transplantation (RTpre) and during conditioning (RTcond).

Results: We estimated the incidence of relapse in our series in 42.9% (21 patients) with a median of 7 months (2-31) and 12.2% (6 patients) showed continuous progression of the disease after transplant. The median overall survival was 3.2 years (0-12) from diagnosis and 1.4 years (0.1 to 4) from transplantation. Fourteen patients died (28.6%): median of 3 years (0.4 to 8) from diagnosis and 16.5 months (5-38) from transplant. The following table shows the impact factors analyzed in contrast to the frequency of relapse after transplantation and mortality: (* Table).

Conclusions: We estimate that the relapse in our patiens was high and found that factors such as patient age, diagnosis and the use of radiation influence in the relapse and the death of patient. Is necessary maintenance treatments after transplantation in an attempt to maintain the response.

**P956**

**Influence of the conditioning regimen in the outcome of dual umbilical cord blood transplants**

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Introduction: Multiple different factors influence the outcome of HSCT, being the conditioning regimen one of those more relevant. Due to lack of prior evaluation of its influence in our strategy employed in the treatment of high risk neoplastic diseases (co-infusion of umbilical cord blood and purified CD34+ stem cells from a third-party donor, DUCBT), here we show the outcome analysis of our patients regarding their conditioning regimen.

Patients and Methods: We included for evaluation consecutive DUCBT patients treated in 3 institutions until December 2010 and retrospectively analyzed their outcome according to the use of total body irradiation (TBI group) or not (CHEMO group) in the conditioning regimen. TBI group received hyperfractionated 1000cGy with pulmonary shielding combined with different chemotherapies and CHEMO group received only myeloablative chemotherapy combinations, both with ATG, cyclosporine A and steroids as GVHD prophylaxis.

Results: We identified 98 DUCBT, 50 in the TBI and 48 in the CHEMO group (Table 1). The TBI group included younger patients (median 33 *vs* 41 years) and more ALL patients (27 *vs* 5). The CHEMO group had more AML patients (16 *vs* 30). Most patients in both groups were 4-5/6 HLA identical (85%) and there were no differences in CD34+ cells/kg or TNC/kg contained in the grafts (median 0.12 x$10^6$ and 2.47 x107 respectively).

Times to ANC and platelets engraftment were not different between both groups. Global graft failure incidence was low (7%) but it was higher in the CHEMO group (2% *vs* 12.5%). Grade II-IV acute GVHD rates were similar but grade III-IV affected more the TBI group (0% *vs* 10%), causing death in 4 cases. With a median follow-up of 60 months for survivors, 1 year-TRM was higher for the TBI group (45% *vs* 22%) and cumulative incidence of relapse (CIR) was lower in the TBI group (10% *vs* 23%), rendering similar EFS and OS at 5y (44%% *vs* 52% and 48 % *vs* 56%, for TBI and CHEMO respectively). In the subgroup of AML/MDS patients (n=51, TBI 15/CHEMO 36), subtle but non significant differences in DFS (44% *vs* 53%) and in OS (50% *vs* 57%) favour the CHEMO group.

Conclusions: With the limitations of a retrospective analysis and patient selection bias between the groups, global results seem equal between TBI and CHEMO groups in our DUCBT platform. Lower CIR and graft failures favour TBI use but higher TRM penalize its results. Further strategies directed to reduce TRM are needed in this context to improve DUCBT results.

[P956]

Table 1. Patients demographics and clinical evolution.

| | All | TBI group | CHEMO group | p (TBI vs CHEMO) |
|---|---|---|---|---|
| Number | 98 | 50 | 48 | |
| Gender (Male/Female) | 61/37 | 30/20 | 31/17 | 0.64 |
| Age: median (range) | 35 (16-64) | 33 (16-60) | 41 (19-64) | 0.03 |
| CB TNC cells/Kg x 10^7 | 2.47(1.14-5.8) | 2.39 (1.14-4.42) | 2.51 (1.48-5.8) | 0.33 |
| CB CD34+ cells/Kg x 10^6 | 0.12 (.035-0.9) | 0.11 (.04-.59) | 0.14 (.035-.90) | 0.12 |
| Follow up time (months) | | | | |
| In all patients, median (range) | 11 (0.2-144) | 12 (0.2-144) | 10 (0.4-101) | 0.32 |
| In survivors, median (range) | 60 (0.2-144) | 78 (2.8-144) | 26 (0.4-101) | 0.01 |
| Time to ANC recovery (P50) | 12 (9 -36) | 11(9-36) | 12 (9-33) | 0.50 |
| Time to platelet recovery (P50) | 38 (9-120) | 40 (14-96) | 39 (9-98) | 0.91 |
| Time to full CB chimera (P50) | 48 (9-200) | 42 (15-81) | 54 (9-186) | 0.86 |
| OS (% 1, 3, 5 years) (KM) | 56, 48, 47 | 55, 48, 46 | 57, 51, 47 | 0.62 |
| DFS (% 1, 3, 5 years) (KM) | 49, 45, 44 | 53, 47, 44 | 57, 48, 44 | 0.79 |
| TRM % 100 days | 28% | 28% | 15% | 0.03 |
| TRM % 1 year | 35% | 45% | 22% | 0.03 |
| Acute GVHD II-IV | 20 (20.4%) | 13 (26%) | 7 (14.6%) | 0.21 |
| Acute GVHD III-IV | 5 (5%) | 5 (10%) | 0 (0%) | 0.056 |
| Chronic GVHD (extensive) | 28% (8%) | 5/36 (14%) | 3/41 (7%) | 0.71 |
| Graft Failure | 7/98 (7%) | 1/50 (2%) | 6/48 (12.5%) | 0.057 |
| Relapses | 16.3% | 10% | 23% | 0.072 |

Legend: ANC: absolute neutrophils counts, CB: cord blood, DFS: disease free survival, GVHD: graft vs host disease, P50: percentile 50%, OS: overall survival, TRM: Transplant related mortality.

**P957**

**Validation of the EBMT risk score in patients receiving allogeneic haematopoietic stem cell transplantation at a single centre in Japan**

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Background: To predict the outcome in patients who received allogeneic stem cell transplantation (HSCT), several methods for assessment of risk score have been reported. In this study, we assessed the usefulness of the European Group for Blood and Marrow Transplantation (EBMT) risk score in patients receiving HSCT at a Japanese single center.
Patients and Methods: The EBMT risk score was retrospectively evaluated in consecutive patients with hematologic disorder who underwent HSCT between 2000 and 2010. The patients were divided into four risk groups according to the EBMT risk score: low risk (LR, score 0-2), intermediate risk-1 (IR-1, score 3), intermediate risk-2 (IR-2, score 4) and high risk (HR, score 5-7).
Results: There were 152 male and 130 female, with a median age of 44 (range, 16-65 years). A median follow-up period was 5.5 (range, 0.2-11 years). The study included 137 patients with AML, 72 ALL, 35 MDS, 19 CML, 9 aplastic anemia, 5 multiple myeloma, and 5 others. HSCT following myeloablative conditioning (MAC) and reduced-intensity conditioning (RIC) for hematologic malignancies were carried out in 205 and 68 patients, respectively. The 5-year overall survival (OS) of LR (n = 71), IR-1 (n = 70), IR-2 (n = 71) and HR (n = 70) group was 74, 59, 42 and 25%, respectively (P < 0.001). The transplant-related mortality at 5-year was 15% in LR, 24% in IR-1, 29% in IR-2 and 36% in HR group (P = 0.041). The 5-year cumulative incidence of relapse was 15% in LR, 15% in IR-1, 36% in IR-2 and 42% in HR group (P < 0.001). On univariate and multivariate analyses for individual risk factors consisting of scoring system, disease stage, donor type, and donor-recipient sex combination were significantly associated with the outcomes, whereas age and time interval from diagnosis to transplant were not significant predictors. In the subgroup analysis, the prognostic value of the EBMT risk score was confirmed in patients with MAC, but not RIC (5-year OS were 85%, 36%, 53%, and 36%, respectively, for patients with LR, IR-1, IR-2, and HR group, P = 0.101).
Conclusions: The results suggest that the EBMT risk score is a useful tool predicting the transplant outcome for patients with MAC and is beneficial for stratifying patients in clinical studies, although reassessment of variables might be necessary to predict outcome in adult patients with RIC.

**P958**

**Factors influencing outcome and incidence of late complications in children who underwent allogeneic haematopoietic stem cell transplantation for haemoglobinopathy: a single-centre analysis**

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Background: Hematopoietic stem cell transplantation (HSCT) remains the only potentially curative treatment for severe hemoglobinopathy (HGP).
The goals post allo-HSCT include immediate survival and complete recovery of the overall health status. It is common to define as late complications (LCs) all events occurring beyond 3 months post-HSCT and separate them into delayed (3 months-2 years), and late (2-10 years) post-HSCT.
Objectives: The aims of this study are to retrospectively analyze prevalence, factors influencing occurrence and prognosis of LCs post-HSCT for HGP at the Rambam Health Care Campus, Haifa, Israel between 2000 and 2011.
Patients and Methods: Between September 2000 and September 2011, 47 patients (Pts) (21 males, 26 females) who had survived more than 2 years (y) post-HSCT for HGP were retrospectively reviewed. 43 pts were diagnosed with beta thalassemia major and 4 pts with sickle cell disease (SCD), mean age at HSCT was 7.7 y (1.1-32 y) and the mean follow-up was 7.1 y (2-11.6 y), 24 pts in class III, 17 class II and 6 class I. 11 pts were splenectomized, mean ferritin level 3022 ng/ml (350-10900), 7 pts post-second HSCT for transplant rejection

(n=6) or aplastic anemia (n=1). Age at HSCT iron overload (IO), class, second HSCT, stem cell source, conditioning regimen, cGVHD, drugs, sex-mismatched and splenectomy were analyzed as risk factors.
Results: Among the 7 pts post- second HSCT, PGF was found in all mature females (3) and in 2 of 3 mature males, FSS in all pts. No significant difference between the incidence of the other LCs.
Among the 11 splenectomized pts, 2 (18%) died of sepsis during treatment of cGVHD.
Conclusion: LCs post HSCT for HGP are common, endocrine LCs are the most frequent and the etiology is multifactorial with

**[P958]** **Table 1**

| Patients total, n=47 | Splenectomized, n=11 | Not splenectomized, n=36 |
|---|---|---|
| LCs-related mortality, n(%) | 2(18) | 0 |
| Elevated transaminase levels, n(%) | 8(73) | 12(33) |
| Endocrine LCs, n (%) | 10(91) | 11(30.5) |
| CGVHD, n (%) | 7 (64) | 5 (13) |
| Stem cell source, n(%): PBSC | 5(45) | 20(55.5) |
| BM | 6(54.5) | 11(30.5) |
| UPBSC | --- | 2(5.5) |
| CB | --- | 2(5.5) |
| UCB | --- | 1(3) |
| Class III, n (% of total) | 8 (73) | 14(39) |
| Class I-II, n (% of total) | 3(27) | 22(61) |
| Mean age at HSCT | 13.2 | 7.3 |
| Mean ferritin levels (ngr/ml) | 4900 | 2560 |

**Table 2**

| | Late complication | Nb pts (% of total) | Risk factors |
|---|---|---|---|
| Mortality | Sepsis | 2(4) | Splenectomy,cGVHD, IO, immunosup. therapy |
| GVHD | Chronic | 12(27) | Stem cell source splenectomy,age |
| Endocrine | Primary gonadal failure (PGF)-mature females | 16(80) | IO, class III, Chemotherapy, age Corticosteroid (CS) therapy, cGVHD Splenectomy |
| | PGF-mature males | 4(36) | |
| | Final short stature (FSS) | 14(52) | |
| | Hypothyroidism | 5(11) | |
| | Diabetes mellitus | 4(9) | |
| | Hypoadrenalism | 2(4) | |
| Hematologic | Mild anemia | 3(7) | Splenomegaly, CsA, thalassemia trait |
| | Leukopenia | 1(2) | |
| | ITP | 4(9) | |
| | Aplastic anemia | 1(2) | |
| Neurological | Seizures | 3(7) | CsA, SCD |
| Respiratory tract | Bronchiolitis obliterans, reactive airways disease | 5(11) | CGVHD Infection |
| Skeletal | Avascular necrosis | 2(4) | CS therapy PGF, IO |
| | Osteoporosis | 10(22) | |
| Ocular | Cataract | 4(9) | CS therapy CGVHD |
| | Corneal perforation | 1(2) | |
| | Keratoconjunctivitis | 1(2) | |
| Cardiovascular | Arrhythmia | 5(11) | IO |
| Liver | Elevated transaminase levels | 20(44) | IO, class, cGVHD, HB react. |
| | Hepatitis B reactivation | 2(4) | |
| Dental | Caries, dental plaque | 7(15) | IO, drugs, cGVHD |

iron overload, class, splenectomy, age, cGVHD and CS treatment. Splenectomized pts were more affected.
CGVHD associated with splenectomy may increase the incidence of LCs leading to an increase in morbidity and mortality. These data may help in life long follow-up in order to limit, detect and treat any LC.

**P959**

**The quality of life following haematopoietic stem cell transplantation, retrospective study of Czech transplant centres, the first interim analysis**

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Background: Haematopoietic stem cell transplantation (HSCT) is standard therapeutic procedure in many haematological diseases. HSCT has curative potential for many patients but at the same time it can significantly worse the patient's life. Quality of life was studied in a series studies of transplants patients. We decided to perform this analysis on the whole group of patients who underwent transplant in the Czech Republic.
Methods: Our project has a retrospective and a prospective part. We present the first data from the retrospective part. There were 5130 autologous and 2260 allogeneic stem cell transplant resp. (ASCT and AlloSCT resp.) performed in years 1986 – 2010. 58% patients were recognized to be alive longer than one year after HSCT, 39% dead and 3% unknown. The standard FACT-G test was used, we have added couple of question employment status and possible recommendation of this procedure to the other patient.
Results: During January 2011 to December 2011 we have received 708 questionars from patients, who underwent HSCT 1-21 (mean 4,6) years ago. The patients are divided into two groups: after ASCT (471 pts) and after AlloHSCT (237 pts). Most frequent diagnosis leading to transplantation were acute leukemia (116) and chronic myeloid leukemia (32) in AlloHSCT and myeloma (183) and lymphoma (226) in ASCT. More than one half of the patients after AlloHSCT (51%) considered their current health status and QOL as good to excellent, and 81% would recommend transplantation to their friends. Only one quarter (24%) of AlloSCT pts were able to return to their jobs. In ASCT group 80% pts reported their current QOL was the same or better than before transplantation, 40% considered their current health status and QOL as good to excellent, 35% were able to return to their jobs and 67 % would recommend transplantation to their friends. These preliminary results of QOL will be correlated to the current status of the patient´s disease.
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**P960**

**Self-reported long-term quality of life of patients with an ICU-admission during treatment for haematological malignancies**

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Introduction: The long-term consequences of an intensive care unit (ICU) admission for survivors of treatment for a haematological malignancy are unknown. Long-term health-related quality of life (HRQoL) as well as fatigue, cognitive functioning, anxiety and depression were compared for patients that were admitted to the ICU during their treatment for a haematological disease (HaemICU+), with those that were not (HaemICU-) and medical ICU patients without haematological disease (MedICU+).
Patients and Methods: A prospective cross-sectional study was carried out. HRQoL was measured using the short form-36 (SF-36), checklist individual strength (CIS-fatigue), cognitive failure questionnaire (CFQ) and hospital anxiety and depression scale (HADS). At median of 18 months after admission, questionnaires were sent to 143 patients with haematological malignancies (HaemICU+ and HaemICU-), treated between February 2008 and February 2010. Data of MedICU+ patients were retrieved from 915 medical ICU patients admitted between February 2008 and February 2009.
Results: 27 (79%) of 34 HaemICU+ patients (mean age 53±14 years), and 93 (85%) of 109 HaemICU- patients (54±13 years) replied. Mortality 18 months following admission was higher in the HaemICU+ compared to HaemICU-: 108/142 (76%) versus 234/431 (45%), respectively (P<0.0001). Data were adjusted for relevant covariates (age, gender, APACHE II-score and length of ICU stay) and matched with MedICU+ patients (n=149, 57±17 years). Hospital length of stay was significantly longer for HaemICU+ (median 33 days [interquartile range 25-42], compared to the HaemICU- (21 [11-27] days, P<0.001) and MedICU+ (18 days [10-37], P<0.001). Besides a lower level of role-physical functioning in HaemICU+ (P=0.03), no other differences were found of the SF-36, CIS-fatigue, CFQ-total and HADS scores between HaemICU+ and HaemICU- patients. Compared to MedICU+, HaemICU+ patients evaluated their HRQoL on SF-36 similarly, except for a lower aggregated physical component summary (PCS, P<0.0001). Also, CIS-fatigue and CFQ-total score were similar. Data of the HADS was not available for the MedICU+ patients.
Conclusion: ICU admission of patients with haematological malignancies who subsequently survived at least 1 year, had no relevant impact on their long-term quality of life, illustrating that limitations of medical treatment should not be based on an assumed lower long-term HRQoL in hematological patients.

**P961**

**Quality of life in children with blood disorders after allogenic haematopoietic cell transplantation at long-term follow-up. Is chronic graft-versus-host disease accompanied by quality of life impairment?**

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Objective: Quality of life (QoL) is increasingly used as a treatment outcome along with traditional clinical outcomes in pediatric patients after allo-HCT. It's worthwhile that the majority of symptoms experienced by children after one year posttransplant are related with chronic GVHD. The aim of our study was to assess the QoL parameters in children at long-term follow up after allo-HCT and to analyze the impact of GVHD on child's QoL.
Methods: 34 children (girls/boys–15/19; mean age 9 y.o., range: 3 to 19 y.o.) after HCT for acute leukemia (n=23), aplastic anemia (n=3), chronic myeloid leukemia (n=3), Fanconi anemia (n=3) and Wiskott-Aldrich syndrome (n=2) were enrolled in the study. Myeloablative conditional regimen (CR) was used in 17 pts (50%), reduced intensity CR - in 17 pts (50%). Median follow-up after HCT was 25.5 months (range 10-79). For comparison 133 healthy controls matched to survivors by age and gender were included in the study. The PedsQL™ self-report and parent-report forms for corresponding age groups were

used for children QoL assessment. To determine differences between groups we used t-test for independent samples or Mann-Whitney test to compare means or medians. An overall alpha level of 0.05 was used as a cut-off point for statistical significance and statistical tests were two-sided.
Results: Pediatric patients at long-term follow-up posttransplant had lower QoL parameters as compared with healthy children in the age groups: 2-4 y.o, 8-12 y.o and 13-18 y.o. Statistically significant differences were found for physical (64.4 *vs* 80.1) and social functioning (77.5 *vs* 86.7) in 8-12 y.o. patients ($p<0.05$). In the age group 5-7 y.o. QoL parameters were similar in HCT recipients and healthy children. Recipients with cGVHD demonstrated a tendency of worse QoL parameters, as compared with those without cGVHD. Differences were observed for both self-reports and parent-reports. QoL parameters were lower for parent reports than for self-reports.
Conclusion: Children with blood disorders have decreased QoL at long-term after allo-HCT as compared to healthy children in the age groups: 2-4 y.o, 8-12 y.o and 13-18 y.o. The significant impairment was observed for physical and social functioning in the age group of 8-12 y.o. In children with cGVHD QoL parameters are worse than in those without cGVHD. This confirms the importance to monitor and control QoL in pediatric recipients of allo-HCT at long-term follow up.

**P962**
**Intravenous gammaglobulin therapy for neurologic manifestations of chronic GvHD after allogeneic haematopoietic stem cell transplantation**
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Introduction: Neurological manifestations of chronic GVHD are rare and can affect both the peripheral and central nervous system. The chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an unusual but important complication of hematopoietic stem cell transplantation (HSCT) occurring with a frequency of 1-2%.
Material and Methods: We describe a series of 6 pts (AML: 3; CML: 1; AA: 2) who underwent allogeneic HSCT and developed CIDP as part of graft-versus-host disease. The median age 26 years (17-41), and sex-ratio: 0,2 (M/F: 1/5). Median interval from diagnosis to transplant was 6 months (4 to 9). The conditioning regimen consist on a myeloablative chemotherapy only. GVHD prophylaxis associated Ciclosporin (CsA) and Methotrexate. All pts received peripheral SCT from an HLA identical sibling donor. Investigations concern detection of high cerebrospinal fluid protein, sensory-motor demyelinating polyradiculoneuropathy by electrophysiological study and radiological abnormalies by CTscan.
Results: The first neurologic symptoms appear within an average of 23 months (6-56): diplopia, generalized muscle pain, paresthesia, functional impairment of the lower, syndrome of the cauda equina, pyramidal syndrome and posterior cord. Symptoms were observed in 5 pts since immunossuppressive therapy for prophylaxis or GVHD treatment. Four pts had presented CMV infection before the neurological symptoms. Cerebrospinal fluid protein was negative for 4/4 pts. CT brain and/or lumbosacral performed in four pts was normal. Electromyography performed in all pts showed sensory-motor demyelinating polyradiculoneuropathy. The treatment involves injecting intravenous (IV) gammaglobulins at 0.4 g/kg/day dose for 5 days, 4 to 6 cures. Gammaglobulins are associated with continues of ciclosporin and corticosteroids (5 cases). In terms of response: complete regression was obtained in 4 cases (66,6%), a failure is observed in one case, even after the addition of rituximab and one patient died of severe infection after only one cure.
Conclusion: Neurological manifestations in chronic GVHD are rare, but they can have a major impact on the disease course after allogeneic HSCT. Early recognition of neurological complications is important to treat pts properly. IV gammaglobulin, associated to cyclosporine and corticosteroids, appears to be an effective treatment as shown in our series.

**P963**
**Prognostic significance of the inflammatory response during pre-transplant conditioning in allogeneic stem cell transplantation**
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Background: The inflammatory response induced during the conditioning regimen may be related to the outcome in allogeneic stem cell transplantation (SCT). Although previous studies have suggested a role of inflammatory cytokines, the results of these studies have been inconsistent due to highly variable levels of cytokines during the course of transplantation. However, C - reactive protein (CRP) released by hepatocytes in response to IL-6, has proven a sensitive and reliable marker of inflammation in infectious and inflammatory disorders. This study was undertaken to test the hypothesis that the inflammatory tonus expressed as mean CRP levels throughout the conditioning is associated with the outcome in SCT. Moreover, we evaluated associations between clinical baseline parameters and CRP levels.
Patients and Methods: During the period 2000 to 2009 777 patients underwent SCT at the National Danish SCT centre. Of those CRP levels were available from 349 patients (aged 27.3 years (0.3 years– 60.3 years), transplanted for malignant haematological diseases (n=288) or benignant diseases (n=61). Donors included matched siblings (SIB, n=170) and matched unrelated donors (MUD, n=179). A mean of 6.5 (interquartiles range 6-8) CRP measurements were available from day –7 to day 0 from each patient.
Results: The mean CRP level during the conditioning was elevated (27 mg/l) compared to normal range (0-10 mg/l).
Mean CRP levels above normal range were associated with reduced overall survival (OAS, $p<0.0001$), increased risk of relapse ($p=0.001$) and increased treatment related mortality (TRM, $p=0.032$). An analysis stratified of CRP levels revealed increased TRM in the SIB group ($p=0.004$) and increased risk of relapse in the MUD group ($p=0.02$). Mean CRP levels were not associated with the risk of acute GvHD.
A high mean CRP level was associated with high risk leukemia *vs*. standard risk leukemia (21 mg/l *vs*. 33 mg/l, $p<0.0001$), MUD *vs*. SIB (19 mg/l *vs*. 34 mg/l, $p<0.0001$), conditioning with TBI/Cy (29 mg/l) *vs*. TBI/V16 (22 mg/l) and Bu/Cy (23 mg/l) ($p=0.028$) and ±ATG (43 mg/l *vs*. 15 mg/l, $p<0.0001$).
Perspective: Risk of relapse, treatment related mortality and overall survival are associated with a high inflammatory tonus as expressed by CRP measurements during the conditioning. The potential important prognostic information given by this easily obtained analysis should be addressed in prospective studies.

**P964**
**Pre-transplant predictive models for allogeneic haematopoietic stem cell transplantation**
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Background: With the introduction of RIC regimens and the improvements in the supportive care, allo-HSCT is been currently offered to more pts. A balanced risk-benefit approach of candidates for allo-HSCT is the key for maximized chances of cure with acceptable quality of life.

Objective: To compare the potential utility of two models, the PAM (pretransplant assessment of mortality; Parimon *et al*, AIM 2006) and the HCT-CI (HCT comorbidity index; Sorror *et al*, Blood 2005), in our cohort of pts.
Pts and Methods: we retrospectively studied 87 pts (51 male, 36 female) with a median age of 49 years (15-65), who underwent allo-HSCT in our center between January 2007 and January 2011. The patients' baseline diseases were: MM (30%), AML (22%), NHL (12.5%), ALL (10%), HL (4.5%), CLL (4.5%), SMD (4.5%), CML (2.3%), WM (2.3%), AA (2.3%), MF (2.3%), and others (5%). 60% pts received allo-HSCT from HLA identical siblings, and 40% from unrelated donors. 26.4 % pts received RIC regimens. Median and maximum follow-up were 16 and 59 months, respectively. Results: for PAM, 9.2% pts had low risk, 64.4% intermediate risk, 21.8% high risk, and 4.6% very high risk; for HCT-CI, 71.3% pts had low risk, 18.4% intermediate risk, and 10.3% high risk. After allografting, 53% pts had complications, the most frequent being: infections (32%), nephrotoxicity (32%), pulmonary toxicities (9.2%), and hepatotoxicity (9.2%). Only 45% of pts included in low and intermediate levels of PAM score presented any complications *vs* 74% of pts with high/very high risk (p: 0.018). Acute GVHD developed in 36.8% pts, and chronic GVHD in 35%. For PAM score: 29.5% of pts with low/intermediate risk had aGVHD *vs* 63.6% of pts with high/very high risk (p: 0.018). Contrarily, the HCT-CI index was not good predictor of complications or GVHD. Non-relapse mortality (NRM) was 23%. Causes of NRM included infections (50%), hemorrhage (20%), pulmonary toxicities (15%), GVHD (5%), cardiotoxicity (5%), and hepatic toxicity (5%). The PAM score effectively risk-stratified the pts for NRM: 0%, 18%, 42%, and 50% in the low, intermediate, high and very high risk groups, respectively, showing a clear distinction by categories (p<0.05). However, we found no impact in NRM when using the HCT-CI model. The correlation between both indexes was poor (see Figure 1).
Conclusions: In our series of pts, risk groups based on the PAM score provided much better discrimination of post-HSCT complications and NRM than the HCT-CI model.

**P965**

**Pre-transplant low serum albumin levels may be associated with poor survival in patients who underwent autologous haematopoietic stem cell transplantation**

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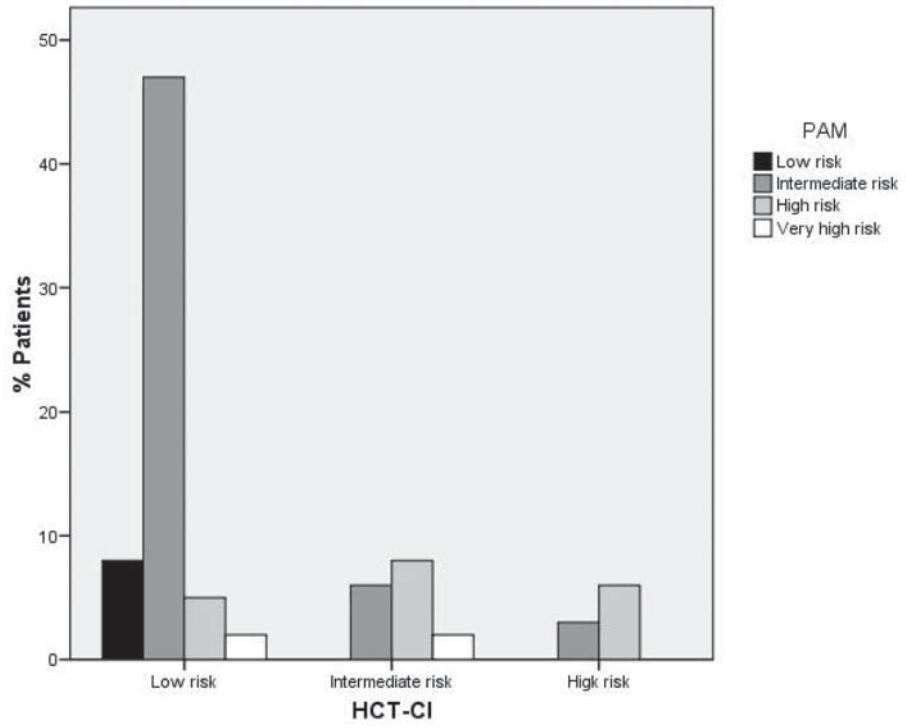
Objectives and Aim: Serum albumin level is mainly a marker of nutritional status in both healthy subjects and patients with malignancies. Our objective was to investigate the association of pre-transplant serum albumin levels with prognosis in autologous HSCT recipients.
Materials and Methods: We retrospectively analysed 106 patients' data who had undergone autologous HSCT diagnosed with multiple myeloma, Hodgkin Lymphoma and Non-Hodgkin Lymphoma. Serum albumin, phosphorus, D-dimer and uric acid, CD34+ cell count, BMI, presence of neutropenic fever of 106 patients were evaluated. The patients' data were obtained from the file records.
Results: 33 (31.1%) of the patients were female, and 73 (68.9%) were male. The diagnosis of the patients were; Hodgkin Lyphoma (33 patients, 31.1%), non-Hodgkin lymphoma (34 patients, 32.1%), and multiple myeloma (39 patients, 36.8%). The median age was found 42 years (min-max: 17-67). Univariate and multivariate analysis showed that low albumin levels (<3.2 g/dL) were associated with decreased overall survival (OS) and disease-free survival (DFS) compared with normal albumin levels (p=0.016 and p=0.001 respectively). A higher risk of death was observed in low-albumin group (HR=2.69, CI:1.17-6.24, p=0.016 for OS and HR=2.69,CI:1.17-6.24 p=0.001 for DFS). Cox regression analysis showed that; low albumin levels were associated with increased risk of relapse but this was not statistically significant (HR:0.97 with %95 CI:0.28-3.32, p=0.96). Serum uric acid, D-dimer, phosphorus levels, CD34+ cell count, BMI, presence of neutropenic fever, age and gender were not associated with poor OS and DFS (p>0.05).
Conclusion: Pre-transplant serum albumin levels may be associated with poor outcomes in patients who had undergone autologous hematopoietic stem cell transplantation regardless with primary diagnosis.

**[P964]**



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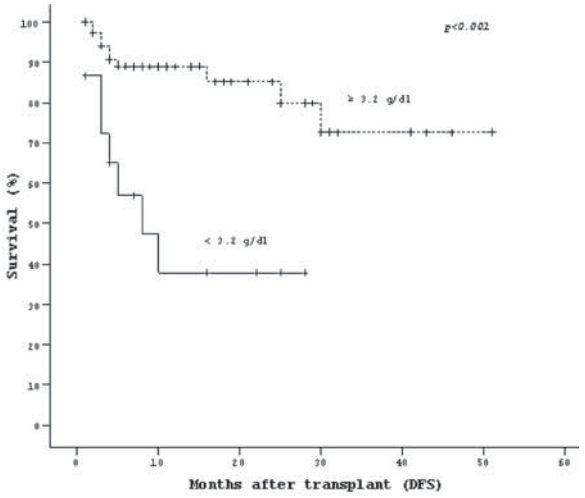


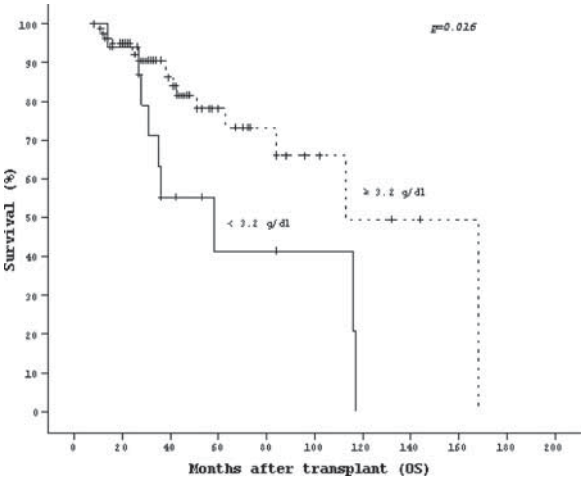


**P966**
**Intramyocardial autologous peripheral HSCT in AML patient with heart failure after alloHSCT**
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Background: Heart failure (HF) after alloHSCT affects up to 5% patients in 5 years and 9% patients in 15 years after the procedure. Intracardial HSCT could lead to improved ventricular remodeling, better exercise tolerance, and potentially improved survival. We report a case of a patient successfully treated with intracardial autoHSCT 18 years after alloHSCT.
In 1990, a 35-year old male patient was referred for AML. He received induction course and, subsequently, 12 maintenance courses of DAT. His sister was a matched donor and alloHSCT was performed in 1992. He was prepared with Cy/TBI and received methotrexate and cyclosporin for GvHD prophylaxis. Grade I skin aGvHD was treated with topical steroids. Otherwise, posttransplant course was unremarkable and he remains in continued remission.
In 2005, he developed HF attributed to a combination of ischemic and post HSCT cardiomyopathy. Because of HF progression, intracardial autoHSCT was proposed. Before the procedure, full donor chimerism was confirmed. In September 2010, 55.9x$10^6$ CD34+ cells were collected after filgrastim mobilization, and after immunoselection, 11.5x$10^6$ CD34+ cells were delivered intramyocardialy. Posttransplant course was unremarkable. Results of cardiovascular parameters before and after the procedure are presented in Table 1.
Discussion: Anthracyclines are well known cardiotoxic agents. Cyclophosphamide and TBI are similarly associated with cardiotoxicity, while cyclosporin and steroids are associated with hypertension and hyperlipidemia. All the mentioned factors can contribute to HF either directly or through increase of risk factors. HF has a progressive course and at some point, pharmacological therapy fails. Intracardial autoHSCT can improve both ejection fraction of left ventricle and overall performance.
Conclusion: Intramyocardial autoHSCT was performed for progressive HF in AML patient after alloHSCT. The patient's condition improved and he is still in 1st remisison more than 1 year after procedure.

[P966]

Table 1: Cardiovascular parameters before intracardial HSCT and during 1st year follow up.

| Time (months) | NYHA class | 6-minute walk test (m) | ProBNP (ng/l) | Heart ejection fraction (%) |
|---|---|---|---|---|
| 0 | II | 410 | 4560 | 20 |
| 1 | II | 431 | | 20 |
| 4 | II | 427 | 7114 | |
| 8 | II | 482 | 3850 | |
| 12 | II | 484 | 5750 | 25-30 |

## Paediatric Issues

**P967**
**Earlier platelet recovery with once-daily busulfan dosing in myeloablative conditioning regimen in paediatrics**
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We studied the pharmacokinetic (PK) profile and outcomes of IV busulfan (BU) in children receiving myeloablative conditioning regimens for first allogeneic hematopoietic stem cell transplantation. A cohort of 39 patients was studied comparing single daily dose BU as part of reduced-toxicity conditioning (RTC-4BU) transplantation with 4 divided doses daily standard

regimen (16BU) both given over 4 days. Patients in 4BU group (n=22) received a BU test dose for PK studies, followed by four treatment doses along with fludarabine, ATG and 400Gy TBI. Patients in the 16BU group (n=17) received first BU dose (0.8-1 mg/kg/d based on age) and PK studies drawn followed by remaining 15 doses along with cyclophosphamide ± etoposide but no TBI. In both groups treatment dose was adjusted to target an area under the curve (AUC) of 4000 microM*min per day. Patients in both groups attained a similar AUC with the test dose (952-963 micrM*min). Final regimen dose AUC attained was 3754 microM*min in the 4BU group and was not measured in the 16 BU group. All patients in the 4BU group engrafted while there were two cases of graft failure (cord blood stem cell source) in the 16BU group. Median time to neutrophil engraftment was comparable in the 4BU and 16BU group (12 *vs* 14 d). Time to platelet count >20K and>50K varied significantly from a median of 14d and 15d in the 4 BU group to 30d and 33d in the 16BU group. OS, EFS were comparable with no significant difference in relapse, GVHD and 100d mortality. We conclude that single daily BU administration is a safe, effective and easier method of dosing for myeloablative conditioning regimens prior to HSCT with potential benefit from earlier platelet engraftment.

**P968**

**Treosulfan, fludarabine and alemtuzumab conditioning for haematopoietic stem cell transplantation in children with chronic granulomatous disease: experience in three centres**

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Chronic Granulomatous Disease (CGD) is a rare primary immunodeficiency predisposing to infection and inflammation. Haematopoietic Cell Transplantation (HCT) is curative, but there is significant morbidity and mortality associated with conventional Busulfan/Cyclophosphamide containing myelo-ablative conditioning regimens. We examined results in a series of patients who received treosulfan and fludarabine as chemotherapy. Sixteen patients received treosulfan 42 g/m$^2$ or 36 g/m$^2$ (1), fludarabine 150 mg/m$^2$ with Alemtuzumab (15), prior to HCT for CGD between 2009 and October 2011 in 3 centres (Newcastle upon Tyne 8, Great Ormond Street Hospital London 5 and Prague 3).

Median age at transplant was 9.3 years (range: 18 months to 15 years). 9 had previous colitis and 5 previous fungal infection. Donors were: unrelated (15) and 1 matched sibling donor. Stem cell source was: PBSC (12), BM (4). Median follow up is short: 15 months (range: 2 to 30).

Overall survival was 87.5%. 2 patients died: 1 was 23 months post transplant with severe GVHD and the other day +1 following MSD transplant in a child with severe inflammatory complications, colitis, fungal and mycobacterial disease. 9 had GVHD, only 1 > grade II. One patient had graft loss, 1 died prior to engraftment and the patient who died from GVHD had 100% donor chimerism.10 are alive and well with 100% donor myeloid chimerism. The 3 from Prague are also alive and well with either 50-60% neutrophil oxidative burst or myeloid chimerism.

Increased use of PBSC may favour improved donor myeloid chimerism and with the use of Alemtuzumab the incidence of significant GVHD was not increased.

Long-term follow up is required to determine the gonadotoxic effects of this approach in comparison to Busulfan containing regimens, but the combination of Treosulfan, Fludarabine and Alemtuzumab is an ideal choice of conditioning for HCT in CGD, associated with good myeloid engraftment and low regimen related toxicity.

**P969**

**Clinical scale generation and functional assessment of cytokine-induced killer cells against acute leukaemia and soft tissue sarcoma**

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Objectives: Allogeneic stem cell transplantation (allo-SCT) has become an important treatment modality for patients with high risk acute myeloid leukemia (AML) and is also under investigation for soft tissue sarcomas. The therapeutic success of allo-SCT is still limited by relapse. Adoptive donor lymphocyte infusions, based on minimal residual disease status, using IL-15-expanded cytokine-induced killer (CIK) cells or CIK cell products with reduced alloreactive-potential, i.e. irradiated and CD56-enriched CIK cells may prevent relapse without causing graft versus host disease (GvHD). To generate preclinical data we studied anti-leukemic- and anti-tumor-potential of CIK cells and CIK cell products *in vivo*.

Methods: Immunodeficient mice were injected intravenously with human AML cell lines THP-1, SH-2 and primary human AML cells or with human rhabdomyosarcoma (RMS) cell lines RH41 and RH30 at minimal doses required for leukemia or tumor engraftment. Mice were randomly assigned for analysis of CIK cell treatment. Organs of mice were analyzed by flow cytometry and quantitative polymerase chain reaction (qPCR) for engraftment of malignant cells and CIK cells. Potential of CIK cells to induce GvHD was determined by histological analysis.

Results: In spite of delayed CIK cell expansion compared with malignant cells, CIK cells injected once at effector to target cell (E:T) ratios of 1:1, 1:2.5, 1:5 or 1:25 were sufficient for significant reduction or elimination of RH41, primary AML, SH-2 or RH30 cells, whereas against fast-expanding THP-1 cells an E:T ratio of 250:1 was needed to achieve comparable results. Mice injected with SH2, primary AML or RH30 cells, which once received conventional CIK cell treatment or monthly injections of CIK cell products, showed an increased disease free survival (DFS) compared with untreated controls. However, DFS was not markedly improved in SH-2-injected mice treated with irradiated CIK cells. Flow cytometric- and PCR-based detection of malignant cells showed that a one-time treatment with conventional CIK cells resulted in the lowest degree of malignant cells compared with repeated injections of CD56-enriched or irradiated CIK cells. Histological analysis of GvHD-targeted organs showed minimal alloreactivity after CIK cell treatment.

Conclusion: In conclusion, our data demonstrated that IL-15-activated CIK cells and even CIK cell products have potent cytotoxic capacity against AML and RMS cells without causing GvHD.

**P970**

**Haematopoietic stem cell transplantation experience of a military medical academy, paediatric transplantation centre**

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Hematopoietic Stem Cell Transplantation (HSCT) in our centre has been performed since 2001 with an average 10 transplants per year. A total number of 83 patients received 87 transplants. The most common indications for allogeneic hematopoietic stem cell transplantation (allo-HSCT) were thalassemia major, acute lymphoblastic leukemia, acute myeloid leukemia and Non-Hodgkin lymphoma. Other allo-HSCT indications were osteopetrorickets, Fanconi anemia, myelodysplasia, chronic myeloid leukemia, severe combined immunodeficiency (SCID), hemophagocytic lymphohistiocytosis, aplastic anemia, Hodgkin lymphoma, langerhans cell histiocytosis, juvenile myelomonocytic leukemia, and Wiskott-Aldrich syndrome. Autologous HSCT (auto-HSCT) was performed mostly for neuroblastoma

and Hodgkin lymphoma. Other indications for auto-HSCT were Ewing sarcoma, acute myeloid leukemia, langerhans cell histiocytosis, primitive neuroectodermal tumor, Wilms tumor and germ cell tumor. The median age of the patients was 8 years (0.5-18 years), with 48 (57%) of patients were male and 35 (43%) were female. Allogeneic was %66 (57) of all transplants while autologous was %34 (30). Of the autologous %3.3 (1) was bone marrow derived, %93.4 (28) from peripheral blood stem cells, and %3.3 (1) was combined transplants. Of the allogeneic transplants, %86 (49) were bone marrow and %14 (8) were peripheral blood stem cell transplants. Of the allogeneic grafts, %89 (51) were taken from matched family members, and %11 (6) were haploidentical. Myeloablative conditioning was given, except the two patients with SCID. Complete hematopoietic engraftment was achieved in 78 (%90) transplants with the median time for neutrophile engraftment was 18 days (9-48 days). Methotrexate, cyclosporine and methylprednisolone or different combinations were used for Graft-versus-host disease (GvHD) prophylaxis. Fifteen patients developed aGvHD while 3 developed cGVHD. Among 15 patients with aGvHD 2 patients died, both of whom were grade IV aGvHD. The 3 patients with cGvHD are alive. The median follow-up period for survivors was 33 months (1–287 months). 57 patients are alive (%68) and 26 patients died (%32). The leading cause of death was persistence or relapse of the underlying disease accounting for 55% of deaths within 100 days and 70% of deaths within 1 year of transplant.

**P971**

**Paediatricallogeneic stem cell transplantation: experience from developing world**

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Introduction: There is lack of data for pediatric allogeneic stem cell transplant (allo-SCT) from a developing country like India. We describe here our experience of allo-SCT programme at Sir Ganga Ram Hospital, a tertiary hospital in North India.
Patients and Methods: A retrospective analysis of all children who underwent allo-SCT from Jan 2008 to Oct 2011 was done.
Results: A total of 28 children (17 males and 11 females) underwent allo-SCT. The mean age was 11.2 years (11 months-18 years). The indications were Thalassemia-13, Aplastic anemia-6, Acute myeloid leukemia (AML)-3, Acute lymphoblastic leukemia-1, Juvenile myelomocytic leukemia-1, Familial Hemophagocytic Lymphohistiocytosis (HLH)-2, Fanconi Anemia-1, Hurler syndrome-1. Donors were HLA-matched sibling-23 and unrelated cord blood-5. The source of stem cells was peripheral blood (PB)-11, bone marrow (BM)-12 and cord blood (CB)-6. One patient received CB and BM from same sibling. 23 patients underwent myeloablative transplants and 5 had reduced intensity conditioning (HLH-2, Thalassemia-1, AML-1, Fanconi-1). Three donor lymphocyte infusions were given to one patient. Neutrophil engrafted in 26 patients at a median of 14 days (range 11 to 44). Four patients had rejection (Thalassemia-2, aplastic-1, Fanconi-1) but all are alive with disease. Eighteen patients (64%) are alive and disease free at a median follow-up of 253 days (65-591 days). Day-100 mortality was 3/28 (11%). Cause of death was Graft *vs.* Host Disease (GVHD)-1, Veno Occlusive Disease-1 and Candida meningitis-1. Three patients died post day-100 due to relapse-1, cytomegalovirus-1 and aspergillus-1. Engraftment syndrome was seen post CB transplant in one child. Acute GVHD was seen in 12 patients (PB-8, CB-1, BM-3), grade III-IV was seen in 4 patients (PB-4). Chronic GVHD was seen in 6 /28 (source BM-1, PB-4). Massive bleeding (gastro-intestinal-2 and pulmonary-1) occurred in three children. Two patients developed sinusoidal obstruction syndrome. Among 5 unrelated CB transplant patients, 4 engrafted. One with AML relapsed at 184 days and died, another with HLH who had received campath based conditioning died of cytomegalovirus infection at day 154 days and one with thalassemia had rejection but is alive post autologous transplant. Two patients are alive and disease free (Thalassemia-1, Hurler-1).
Conclusion: Our results are encouraging and give hope to many children who need allogeneic stem cell transplant in the developing world.

**P972**

**Allogeneic haematopoietic stem cell transplantation for childhood myelodysplastic syndrome**

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Background: Myelodysplastic syndrome (MDS) is very rare in children than in adults. Although the optimal treatment for children with MDS without matched sibling donor (MSD) is presently undetermined, hematopoietic stem cell transplantation (HSCT) using alternative donors should be considered for severe cytopenia. We analyzed the result of allogeneic HSCT for children with MDS in a Korean single center. It is the first report about the outcome of HSCT for Pediatric MDS in Korea.
Methods: Between November 1997 and June 2011, 16 children with MDS underwent allogeneic HSCT. These patients had refractory cytopenia (RC) (n=7), and refractory anemia with excess blasts (RAEB) (n=9). Median age was 8.9 years (range 1.6-20.3). Cytogenetic analysis revealed that ten had a normal karyotype, six had miscellaneous chromosomal changes, but nobody had monosomy 7. Six patients were grafted from MSD, another 6 patients from mismatched unrelated donor, 3 patients from umbilical cord blood, and one from haploidentical related donor.
Results: Sustained neutrophil engraftment was achieved in 11 patients after first transplantation. One patient died of subarachnoid hemorrhage on day 1, and 4 patients experienced graft failure; all patients were regrafted from haploidentical related donors. With a median follow-up of 6.0 years (range 0.1-14), the cumulative incidence of grade II-IV acute graft-versus-host disease (GVHD) was 0.27, and extensive chronic GVHD was 0.12. The cumulative incidence of treatment related mortality and relapse was 0.19 and 0.20. The Kaplan-Meier estimates of overall survival and event-free survival at 5 years were 0.65 and 0.49. Graft failure was associated with the diagnosis of RC (RR 3.67, 95% CI 1.4-9.6, P=0.026) and HLA-mismatched transplant (RR 2.75, 95% CI 1.3-6.0, P=0.05). Over grade 2 acute GVHD increased the risk of extensive chronic GVHD (RR 6.5, 95% CI 1.8-23.2, P=0.05), and extensive chronic GVHD significantly affected OS (P=0.001).
Conclusions: Despite most of patients with RC received myeloablative conditioning including busulfan and cyclophosphamide, they showed high engraftment failure rates, especially in mismatched transplants. However, all of them were successfully regrafted form haploidentical related donors, and showed comparable treatment outcome. This study demonstrated that haploidentical HSCT could be successfully used for graft failure of pediatric MDS.

**P973**

**Haematopoietic stem cell transplants for malignant blood disorders – Results from a single paediatric centre**

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Haematopoietic stem cell transplants (HSCT) is the only cure for children with high risk/relapse acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) since 1970's. In SE Asian population, matched sibling remains the main source of stem cells. Unrelated matched donors for minority races are often difficult to find from international bone marrow registries. Unrelated cord blood is an

[P972]

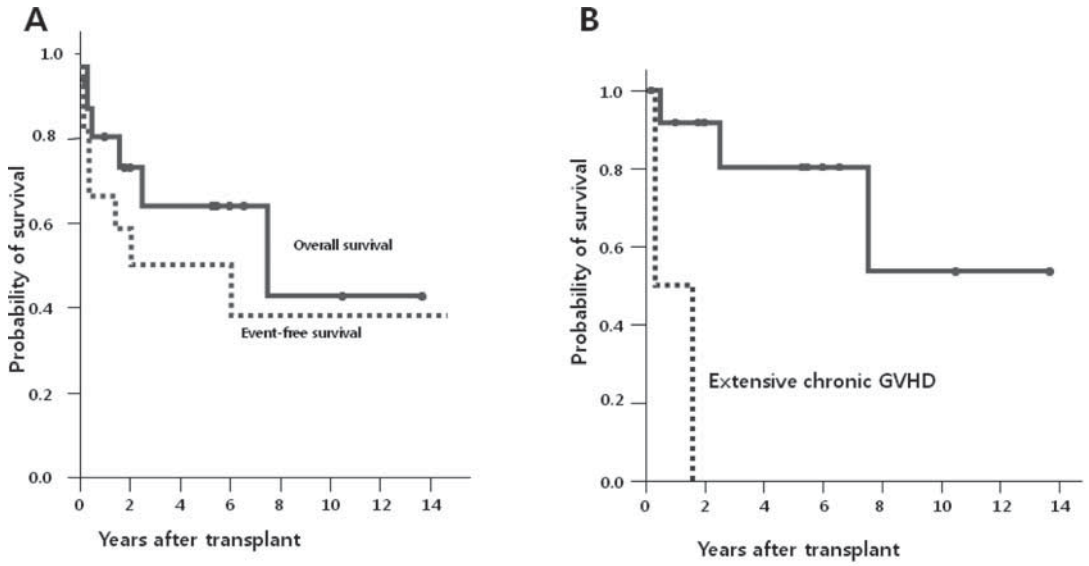


Figure 1. (A) Kaplan-Meier estimates of overall survival and event-free survival for 16 patients with MDS. (B) Overall survival was significantly affected by the presence of extensive chronic GVHD ($P$=0.0013).

alternative source of stem cells in a multiracial population like Singapore. We describe our results for children from a single transplant center.

Methods: We performed allogeneic HSCT for 55 children with leukemia over a period 12 years from May 1998 to Dec 2010. There were a total 56 HSCT, one patient had 2 transplants, 2nd HSCT using MUD after relapse of leukemia from the first unrelated cord blood transplant (CBT). During this period 31 (55%) MSD, 3 (5%) related cord, 5 (10%) MUD and 17 (30%) unrelated cord blood transplants were performed. The underlying conditions were 27 (49%) for ALL,18 (33%) for MDS/AML and 10 (18%) for CML/CMML. Conditioning regimes consisted of myeloablative busulphan and cyclophosphamide (CPA) for AML/CML and TBI +CPA with testicular/CNS ± RT boost for ALL. ATG were added in unrelated sources of stem cells. Graft *vs* host disease (GVHD)prophylaxis consisted of cyclosporin ±methotrexate ± methylprednisolone.

Results: All engrafted well except for one chlid had graft failure after unrelated CBT for relapse ALL but he remained disease-free 6 years post transplant. TRM was 14% (8/55); 3 from infection, 2 from pneumonitis, 1 AGVHD with CMV infection, 2 chronic GVHD (lung/liver). 2 died from late effect of transplant; 1 had brain tumor (PNET) died 8 yrs later and 1 died of chronic lung disease 5 yrs post transplant. Both received TBI in conditioning regime.The median neutrophil engraftment were D+16 (range D+11 to D+27) for BMT and D+26 (range +13 to +46) for CBT. The median platelet engraftment were D+22 and D+35 for BMT and CBT respectively. 2 yrs OS were 65%, 71% while 5 yrs OS were 61% and 55% for BMT and CBT respectively. 2 yrs EFS were 54% and 51% and 5yrs EFS were 45% and 44% for BMT and CBT respectively.

Conclusion: Our results showed that cord blood can form at least 30% stem cell source. This is an important alternative source of stem cell especially in our population with diverse racial groups. The outcomes of CBT are comparable with BMT inspite of slower neutrophil and platelet engraftment.

**P974**

**Successful haploidentical haematopoietic peripheral stem cell transplantation in a child with Wiskott-Aldrich syndrome**

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Wiskott-Aldrich syndrome (WAS) is a rare X-linked recessive, primary immunodeficiency characterized by eczema, thrombocytopenia with microplatelets, recurrent infection, and increased susceptibility to autoimmune disease and lymphoreticular malignancies. To date, the only curative therapy for WAS is hematopoietic stem cell transplantation (HSCT). We describe a case of WAS who was conditioned with fludarabine-based regimen and transplanted by using highly-purified CD34+ stem cells from his HLA 2-loci mismatched mother and achieved sustained full donor-type engraftment and immunologic reconstitution. A nine-month-old boy presented with eczema, microthrombocytopenia and recurrent infection was diagnosed as severe WAS (score 5) by flow cytometric and mutation analysis. A missence g.5640C>A, c.1455C>A mutation on exon 12 was detected. Because of lacking donor, he underwent haploidentical HSCT from his mother at 20 months of age. He received myeloablative regimen consisting of busulfan (16 mg/kg), cyclophosphamide (200 mg/kg), rabbit anti-thymocyte globulin (10 mg/kg) and fludarabine (160 mg/m$^2$). He also received methylprednisolone for graft-versus-host disease (GvHD) prophylaxis. T cell-depletion and positive selection of peripheral CD34+ stem cells from his G-CSF stimulated mother was performed using the method of immunomagnetic separation (CliniMACS). The number of infused CD34+ cells was 16.11x10$^6$/kg. He achieved full hematopoietic engraftment with the time for neutrophil, platelet, and erythrocyte recovery being 11 days, 14 days and 19 days, respectively. He developed grade II aGVHD on day +12, and grade I cGVHD on day +240, resolution achieved in aGVHD with i.v. methylprednisolone and cyclosporine, in cGVHD with oral prednisolone. He was positive for cytomegalovirus which was detected by PCR in his blood, urine and stool samples on day +6 and resolved with ganciclovir. He is now at 30 months of age, he did'nt have any serious infection and fluorescence

in situ hybridization analysis revealed sustained full donor-type engraftment and flow cytometric analysis revealed immunologic reconstitution. Conditioning with fludarabine-based regimens and transplantation of highly purified, mega-dose CD34+ stem cells from haploidentical parents is a reasonable therapeutic option for children with WAS lacking HLA identical donor.

**P975**

**Haploidentical haematopoietic stem cell transplantation in benign and malign diseases in children: report of six cases from a single-centre**

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Haploidentical hematopoietic stem cell transplantation (haplo-HSCT) has provided an alternative option particularly for those who urgently need an allogeneic transplant. Here, we report the haplo-HSCT results of our centre with G-CSF mobilized, CD34±selected peripheral blood stem cells (PBSC) by using the CliniMACS immunomagnetic selection technology. Six children (osteopetrorickets, myelodysplastic syndrome (MDS), severe combined immunodeficiency (SCID), acute myeloid leukemia (AML) (M4) transformed from Fanconi's Anemia (FA), Wiskott-Aldrich Syndrome (WAS) and relapsed AML) with a median age of 7 years (7 months-13 years) underwent 2- (2), 3- (2), and 5- (2)-loci mismatched-haplo-HSCT from related donors. The recipients received myeloablative conditioning regimen except for SCID patient. The children with hematological malignancies were conditioned with fludarabine-based regimen including total body irradiation. Except for the patients with SCID and relapsed AML, the recipients received different immunosuppressants consisting of methotrexate, cyclosporine and methylprednisolone for graft-versus-host disease (GvHD) prophylaxis. G-CSF alone was used for mobilization at a dose of 10 mcg/kg/day for 5 days. Leukopheresis were performed on the fifth day of the G-CSF therapy. The median number of infused CD34+ cells were 13.17 (5.58–19.07) x $10^6$/kg. Five patients achieved full hematopoietic engraftment with the median time for neutrophil, platelet and erythrocyte recovery being 13 days, 17 days and 23 days, respectively. The patient with osteopetrorickets, MDS and FA+AML died at 4, 5 and 9 months after transplantation due to primary engraftment failure, pulmonary hemorrhage, and sepsis, respectively. The others are currently alive in complete remission at a median follow-up of 14 months (range 2-30 months), but the antibody responses of the patient with SCID remained low only for IgA. Five patients achieved full donor-type engraftment. The patient with WAS developed grade II aGVHD on day +12 and the patient with FA+AML developed grade I gastrointestinal aGVHD on day +60. None of patients developed cGVHD. The results here suggest that haplo-HSCT might provide an opportunity for benign and malign diseases, especially for immunodeficiency syndromes who do not have available donor and urgently need an allogeneic transplant.

**P976**

**Can platelet distribution width and mean platelet volume be markers of platelet engraftment in recipients of allogeneic haematopoietic stem cell transplantation?**

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Platelet distribution width (PDW) and mean platelet volume (MPV) are indices which increase during platelet activation. Accelerated thrombopoiesis is one of the reasons of platelet activation. We hypothesized that PDW and MPV may increase towards the platelet engraftment due to accelerated thrombopoiesis and if so can be markers. We studied the relationship between platelet engraftment time and PDW and MPV values in patients, who underwent allogeneic hematopoietic stem cell transplantation (allo HSCT). Haemogram parameters with PDW and MPV of 20 patients with a median age of 7 years (range, 2-13), who underwent allo HSCT were studied. The parameters on the first day of myeloablative regimen (MR), product infusion date (ID), 6 days (-6BE) and 3 days (-3BE) before platelet engraftment, the day of platelet engraftment (ED) and 3 days (+3AE) and 6 days (+6AE) after engraftment were evaluated. Median time for ED was 17 days (range, 14-32). There weren't significant difference between MR and ID in PDW and MPV values, also no significant difference were present between -6BE, -3BE and ED in these values. But there were significant difference between ID,-6BE,-3BE and ED in PDW (14.6±2.3%; 13.4±1.4%; 13.2±1.4% and 13.2±1.3% respectively; p=0.018) and MPV (6.6±0.9 fl; 7.3±3.3 fl; 7.3±1.2 fl and 8.2±1 fl respectively; p=0.007). There weren't significant difference between ED and +3AE in PDW and MPV. But there were significant difference between +3AE and +6AE in PDW (13.0±1.5% and 14.4±3.4% respectively; p=0.017) and MPV (8.3±0.7 fl and 7.5±1.1 fl respectively; p=0.046). There weren't significant difference between MR and +6AE in PDW and MPV. These results shows that there is a gradually significant increase in MPV and significantly decrease in PDW towards the engraftment, which returns to MR values on +6AE. Activated platelets seem larger in analyzers, independently of the principle used. PDW doesn't increase during platelet distention caused by platelet swelling, while this situation increases MPV. In conclusion accelerated thrombopoiesis towards the platelet engraftment seems to be causing the production of uniformly large, swollen platelets, which may be the reason of significant increase in MPV while decrease in PDW, starting from -6BE until to +6AE, the time that MPV and PDW returns to MR values.

**P977**

**Unrelated umbilical cord blood transplantation in children: a single-centre experience**

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Objectives and Materials: We report the outcome of 42 unrelated umbilical cord blood transplantations (UCBT) performed in Akdeniz University Pediatric Bone Marrow Transplantation Unit between 2005-2011. One patient received double cord blood infusions. Seven patients were treated for hematologic malignancies and 35 patients were for non-malign diseases. Eight patients were suffering from inherited genetic diseases, 8 patients had bone marrow failure, 19 patients had primary immune deficiencies. Median age was 2 years (range, 3 months-17 years) and the median weight was 10.8 kg (range, 2.4- 47.5 kg). The preparative regimen was busulphan based myeloablative in 35 patients. HLA DRB1 high resolution molecular typing was available for 35 CB units and matching between donor and recipient was 2 of 6 in 1 patient, 3 of 6 in 2 patients, 4 of 6 in 6 patients, 5 of 6 in 21 patients and 6 of 6 in 5 patients. The median nucleated cell dose was $10.3^5$x$10^7$/kg (range, 1.5-41.5x$10^7$/kg) and the median CD 34+ cell dose was 2.1x$10^5$/kg (range, 0.7-20.5x$10^5$/kg). Graft versus host disease prophylaxis consisted of cyclosporine and steroids or MMF.
Results: The median number of days to an absolute neutrophil count of 500/μL was 23 (range, 8-66 days). The median time to an untransfused platelet count of more than 20.000/μL was 44 days (range, 20-87 days). Eight patients experienced grade II-IV acute GVHD. The one year overall and event-free survival were 62.4 and 39%, respectively.
Engraftment was achieved in 17 patients, 8 patients had graft failure, 11 patients had autologous reconstitution. rejected and 6 patients died within the first month after transplantation before engraftment. Twenty-three patients were alive at a median follow up of 18 months (range, 2-75 months). The primary cause of death was the infection.
Conclusions: These results suggest that unrelated umbilical cord blood is a viable stem cell source for patients who require urgent transplantation and if the family donor is not available.

**P978**

**Examples of the use of stem cells from cord blood in Poland**

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Introduction: Today, the cord blood (CB) is widely recognized as a source of haematopoetic stem cells (HSC) and Wharton jelly (WJ) - as a novel source of mesenchymal stem cells (MSC). Here we report the first use in Europe, autologous (auto) CB-derived-neurally-committed cells (CBNC), first use of WJ derived MSC from “third party donor” and 11 usage attempts of allogeneic (allo) CB units (CBU) from PBKM SA.

Materials and Methods: From January 2007 to November 2011, PBKM SA received 12 requests concerning stored CBU and one request concerning WJ-MSC from “third party donor”. 11 standard requests for allo transplantations (tx), resulted in 7 allo tx and 4 remaining CB requests were withdrawn due to lack of HLA match. Recipients of allo HSC were the children with: early relapse of acute lymphoblastic leukemia (ALL) - 2, neuroblastoma (NBL) relapse after auto HSC tx - 1, myelodysplastic syndrome (MDS) - 1, Fanconi anemia - 1, Langerhans cell histiocytosis - 1, chronic granulomatous disease - 1. All above listed HSC tx, with the exception girl with NBL were first transplants. In one case, the CBU request regarded regenerative procedure, in a boy with cardiac-arrest induced cerebral ischemia (CI) in a permanent vegetative state, who received three auto CBNC tx. The recipient of WJ-MSC, was a 17 year old girl with MDS and chronic Graft versus Host Disease (cGvHD) after allo tx from matched unrelated donor, who experienced steroid resistant gut GvHD flare. Preparation, freezing and storage of all CBU and WJ-MSC were done in PBKM SA. The conditioning regimens were carried out, according to appropriate protocols. Both, boy with CI auto transplanted with partly pre-labeled by iron oxide nanoparticles CBNC and a girl with MDS and cGvHD, didn’t required conditioning regimen before tx.

Results: All CBU engrafted. No adverse events or abnormal reactions, except for transient increases in body temperature in pt with CI, were observed. The pt with NBL relapsed in 18 month and one pt with ALL relapsed in 11 month after transplantation. Other children remain in complete remission. In the boy with CI, mild functional improvement was found – decreased vegetative state, amelioration of spasticity and nystagmus. The girl with GvHD was successfully treated with 2 infusions of WJ-MSC, achieved full remission and was discharged home.

Conclusion: The course of transplantations confirm that CBU and WJ from PBKM SA are the suitable source of HSCs and MSCs, when are used for the treatment.

**P979**

**Treosulphan as alternative to busulphan for myeloablative and reduced-intensity conditioning regimen of allogeneic bone marrow transplantation for high-risk paediatric patients**

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Indroduction: Pediatric patients with immunodeficiency and severe organ dysfunction from previous infections as well as children with lung, heart and liver injury secondary to metabolic diseases or drug induced are at very high risk for life-threatening adverse events and mortality during and after allogeneic BMT. The standard myeloablative conditioning regimen (Busulphan with Cyclophosphamide or TBI protocol) is associated with severe mucositis, lung toxicity and veno-occlusive disease of the liver and causes significant morbidity in patients with previous organ injury. Treosulphan is an alkylating agent with myeloablative,immunosuppressive and neoplastic effects. It has been used as a conditioning agent recently with promising favorable toxicity profile compared with Busulphan and TBI.

Patients: We describe our experience in using Treosulphan in place of Busulphan for myeloablative and reduced intensity conditioning (RIC) regimen in allogeneic BMT for high risk patients.Five children (3 boys and 2 girls) were included. Two patients, 1 - SCID with respiratory failure and severe lung infections and 1 - ALL with cardiomyopathy and severe previous infections who underwent a second BMT - received RIC including: Treosulphan, Fludarabine and Antithymoglobuline (ATG). Three children had myeloablative regimen including: Treosulphan, Cyclophosphamide and ATG (for unrelated donor). Three patients had immunodeficiency (1 SCID, 2 CGD), 1 - Niemann-Pick type C, and 1 ALL for second BMT. Three patients underwent BMT from bone marrow of matched sibling donor, 1 from an unrelated donor and 1 unrelated cord with 1 minor mismatch in DR.

Results: All patients engrafted donor stem cells. The child with Niemann-Pick syndrome and unrelated cord blood developed disseminated adenoviral infection post engraftment and rejected the donor cells. She is alive 5 months after first BMT. The rest had complete or mixed stable chimerism. No patient developed gastrointestinal or upper respiratory mucositis, veno-occlusive or severe infections. All patients continued enteral nutrition. None received antalgics or morphin.All were given less blood products or antibiotics.

Conclusions: Treosulphan may substitute Busulphan for conditioning before Allogeneic BMT especially for non-malignant diseases, providing favorable acute toxicity profile and good primary engraftment, and could be less toxic for children at high-risk.

**P980**

**Reduced-toxicity treosulfan-based preparative regimen for allogeneic HSCT in children with refractory histiocytic malignancy – single-centre experience**

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) improves outcome of refractory haemophagocytic lymphohistiocytosis (HLH) and refractory Langerhans cell histiocytosis (LCH), but the risk of morbidity and mortality related to myeloablative preparative regimen is high.

The aim of the study was to evaluate the results of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in children with refractory HLH and LCH conditioned for allo-HSCT with reduced toxicity treosulfan-based preparative regimen.

Patients and Methods: Between 2001 and 2011, 3 children with disseminated, multi-organ refractory LCH (2 boys, 1 girl;

8, 12 and 24 months of age) and 8 years old boy with refractory HLH underwent allo-HSCT. The reduced-intensity conditioning regimen applied consisted of treosulfan (3 x 10 g/m$^2$) in combination with etoposide 30 mg/kg and cyclophosphamide 2 x 60 mg/kg (n=2) or with fludarabin (5 x 30 mg/m$^2$) and Melfalan 140 mg/m$^2$ (n=1) in children with LCH, whilst in patient with HLH in combination with fludarabin (5 x 30 mg/m$^2$) and MabCampath (anti-CD52 antibody). Patients with LCH were transplanted from matched sibling donors (MSD) (bone marrow, n=2; cord blood, n=1), a child with HLH received peripheral stem cells from matched unrelated donor. GvHD prophylaxis consisted of CSA for all patients, boy with HLH additionally received mycophenolate mofetil.
Results: All patients engrafted (ANC >0.5 x 10$^9$/L on days 15, 18, 24, 50; platelet count >20 x 10$^9$/L on days 13, 16, 18, 41) with complete donor chimerism at engraftment, and subsequently developed mixed chimerism with graft rejection in 2 of them (24 and 12 months after allo-HSCT). As regimen-related toxicity exlusively mucositis of grade 2 to 3 was seen in 2 patients. Two patients demonstrated reactivation of herpes simplex virus, cytomegalovirus, and EBV. All 4 patients are alive and remain in continuous complete clinical remission at 3, 14, 108, and 121 months after allo-HSCT.
Conclusion: In 4 reported children with refractory LCH and HLH the preparative regimen based on treosulfan at the total dose of 30 g/m$^2$ demonstrated the myeloablative effect sufficient for development of curative immunotherapeutic effect of allo-HSCT and no significant organ toxicity. To achieve stable complete donor chimerism in children with histiocytic malignancy a higher total dose of treosulfan should be administered (36-42 g/m$^2$).

**P982**

**Impact of the use of antithymocyte globulin on cellular immune reconstitution after allogeneic haematopoietic stem cell transplantation**

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Introduction: Graft versus Host disease (GvHD) can severely affect quality of life of patients after allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT). To prevent GvHD, ATG is used during the conditioning regimen, which can have an impact on the immune reconstitution. We performed an analysis of the lymphocytes subpopulations at different time points after allo-HSCT to evaluate the impact of the use of ATG on immune reconstitution.
Materials and Methods: The cellular immune reconstitution after allo-HSCT was studied in 32 pediatric patients, with a minimum survival of 3 months. Evaluations were performed at 3, 6, 9 and 12 months or until death or relapse. Patients with diagnosis of T-cell immune deficiency and patients transplanted after CD34 positive selection of the graft were excluded for this analysis.
ATG Thymoglobulin® in most of the cases at a dose of 10 mg/kg was used in 21 pts and 11 pts did not receive any ATG during the allo-HSCT.
Results: Three months after allo-HSCT, the median lymphocyte count is 597/µL. Within the lymphocytes, the population of CD4+ cells is the most severely affected with a median of 122/µl and only 12% of the patients achieving normal CD4 values for age. Patients who received *in vivo* T-cell depletion have even lower age-related CD4 levels than those who did not receive this kind of immune modulation of the graft (p=0.02). The CD8+ cells are less affected, with normal CD8 counts and NK cells in 62% and 50% of the patients respectively.
At 6 months post allo-HSCT, CD8+ reconstitution as well as NK count is normal in 76% of the pts, but CD4 cells are still severely impaired, with a median count of 265/µL and only 19% of the patients having normal counts.
Nine months after allo-HSCT, 76% of the patients have normal lymphocytes counts, with an improvement in reconstitution of the T-helper cells and normal CD4 counts for age in 60%. At 1 year post allo-HSCT 80% of all patients have normal lymphocytes and T-helpers counts. T-cell suppressors are normal in 90% of the patients and all patients have normal NK cell levels.
Conclusion: It is well known that patients after allo-HSCT with full myeloablative conditioning have a severe immunological impairment. The use of ATG in the conditioning regimen retards the immune reconstitution of T- helpers during the first 3 months after allo-HSCT, putting this patients at higher risk for viral infections. We do not find major differences for T-suppressors cells or NK cells.

**P983**

**Is haematopoietic cord blood transplantation an option for patients with severe infantile osteopetrosis?**

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Introduction: Autosomal recessive osteopetrosis (ARO) is a congenital disorder characterized by impairment of bone remodeling due to osteoclast dysfunction, resulting in impaired bone resorption with decrease of the bone marrow space. Clinical consequences include bone marrow failure and compression of cranial nerves leading to different neurological deficits. Though genetically heterogenous, over 50% of ARO patients have mutations in the TCIRG1 gene, encoding the osteoclast-specific H±ATPase proton pump A3 subunit. So far the only available curative treatment is Allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT). Here we describe the characteristics and outcome of 2 patients with ARO who received Cord Blood (CBU) as grafts.
Patients and Methods: Two patients with confirmed TCIRG1 ARO received allo-HSCT with CBU as graft in our Center. Patient's age, Transplant and CBU characteristics are summarized in the Table 1. Patient 1 received 2 CBU, not as part of double CBU program but due to low viability (30%) of the 1st infused CBU.
Discussion: Patients with osteopetrosis are characterized by having a high risk of graft failure, which has been more notorious using CBU as graft. However, in patients with ARO a prompt allo-HSCT is needed to prevent irreversible neurological impairment. We performed unrelated CBU in two patients lacking a HLA- matched donor. The allo-HSCT was successful in both cases without major complications. This suggest that allo-HSCT with CBU as graft is feasible and should be evaluated in those patients without a good matched donor. The CBU must have a good cellularity and the conditioning regimen should be fully myeloablative, for which we suggest the use of Busulfan (full dose), Fludarabine and Melphalan.

**P985**

**Update on post transplant immune reconstitution after allogeneic stem cell transplantation in paediatric high-risk leukaemia patients**

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Immune recovery post allogeneic stem cell transplantation (HSCT) in young children is poorly defined. Guidelines are being elaborated regarding the monitoring of immune recovery post HSCT, especially in heavily pre-treated patients.
Patients and Methods: We retrospectively analyzed the immune recovery in 7 children with very high risk leukemia

[P983]

Table 1

| | Patient 1 | Patient 2 |
|---|---|---|
| **Histological Characteristics** | Not done | Osteoclast poor |
| **TCIRG1 Mutation** | p.R669X/p.R669X | p.R669X/p.R669X |
| **Age at allo-HSCT** | 8 months | 8 months |
| **Conditioning** | Busulfan po 16 mg/kg<br>Fludarabine 140 mg/m²<br>Melphalan 140/m² | Busulfan iv according to weight<br>Fludarabine 140 mg/m²<br>Melphalan 140/m² |
| **Graft vs. Host prophylaxis** | ATG (Thymoglobulin®)<br>4x 2.5 mg/kg (during conditioning)<br>Cyclosporine A up day -1<br>Prednisone 1 mg/kg/dag (day+1 to day+28) | ATG (Thymoglobulin®)<br>4x 2.5 mg/kg (during conditioning)<br>Cyclosporine A up day -1<br>Prednisone 1 mg/kg/dag (day+1 to day+28) |
| **CBU Characteristics** | **1st CBU**<br>Match 5/6 (B MM)<br>CD34 cells infused<br>2.1 x $10^5$/kg (30% viability)<br>**2nd CBU**<br>Match 5/6 (B MM)<br>CD34 cells infused<br>5,63 x$10^5$/kg | Match 6/6<br>CD34 cells infused<br>9 x $10^5$/kg |
| **Engraftment of Neutrophils >500/µL (day +)** | 30 | 13 |
| **Engraftment of Thrombocytes >50 000/µL (day +)** | 66 | 53 |
| **Donor chimerism** | Full donor (1st infused CBU) | Full donor |
| **Clinical evolution** | Good with bone remodeling. | Good with bone remodeling. |
| **Complications** | Ongoing mild/moderate immune hemolytic anemia, clinical very good with low dose steroids | no complications |
| **Follow-up** | 4 yrs. | 1 yr. |

(4 ALL, 3 AML) with median age 9 years (2-15 yrs), transplanted in complete remission, (CR), CR1 (n=5) or CR 2 (n=2) and surviving >12 months post HSCT. All received myeloablative conditioning. Donors were HLA genoidentical (n=3), haploidentical (n=1), unrelated (n=2), unrelated cord blood (n=1). Total chimerism was <3% in all patients during the follow up period. Lymphocyte subpopulations CD3, CD4, CD8, NK19, were available at 3, 6,12,24,36 months and gamma/delta T, CD45R0, CD45RA at 12, 36 months post HSCT.

Lymphocyte proliferation to mitogens (PHA, Con A, PWM) and antigens (Tetanus, Diphtheria, Streptococcus, Candida, Proteus, PPD) were done at 6,12,24,36 months post HSCT. Antibodies against Tetanus, Diphtheria, Pneumococcal 14, 19, 23, H. influenza were tested 12, 24, 36 months post-transplant.

Results: Lymphocyte subpopulations were normalized between 12-24 months post HCST. Mitogen proliferation was normal at 12 months and antigen proliferation between 12 and 36 months post HSCT. Antibody responses to all the antigens tested were normal at 12 months post HSCT; however, there was a continuing decline in all, especially to pneumococcal antibodies, resulting in non protective titers at 36 months, therefore necessitating booster vaccinations.

Conclusions: We described a limited number of transplanted young pediatric patients with very high-risk leukemia. This small study confirms that for heavily treated leukemia patients, complete immune recovery is achieved 3 years post HSCT. Nevertheless, antibody titers against protein and polysaccharide antigens are very low despite previous vaccinations. These patients should, therefore, receive booster vaccinations at three years post transplantation. We suggest that specific antibody responses be checked yearly in this group of patients.

**P986**

**Diamond Blackfan anaemia: when to transplant?**

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Diamond Blackfan anaemia (DBA) is a rare genetic disease, characterized by proapoptotic erythropoiesis/erythroid aplasia, congenital anomalies and predisposition to cancer. Hematopoietic Stem Cell Transplantation (HCST) is the only definitive treatment for its hematologic manifestations.
Patients and Methods: Two cases of DBA transplanted at an early age. Patients received myeloablative conditioning.
Donors were HLA siblings, negative for the mutations.
Chimerism was done once/month at first 6months, every 3 months thereafter. Lymphocyte subpopulations CD3, CD4, CD8, CD19, NK were available at 6,12, 24 months post HSCT.
Lymphocyte proliferation to mitogens (PHA, ConA, PWM) and antigens (Tetanus, Diphtheria, Streptococcus, Candida, Proteus, PPD) were done at 6,12 months post HSCT.
Antibodies against Tetanus, Diphtheria, Pneumococcal 14, 19, 23, H. influenza were tested at 12 months post-transplant
Results: Patients had significant mitogen proliferation even at 6 months post transplant and normal lymphocyte subpopulations and antibody responses to Tetanus and Diphteria at 12 months post transplant. Both are now transfusion independent with normal ferritin levels and no organ dysfunction.
Conclusions: We described two pediatric patients with DBA anemia transplanted early. Both had mild/moderate transplant related morbidity and adequate immune recovery early after transplantation. Allogeneic sibling stem cell transplantation in young DBA patients without significant iron overload or organ dysfunction seems to be a reasonable alternative to corticosteroid or transfusion therapy and obviates the risk of trilineage hematopoietic failure or hematologic malignancy. The controversy regarding HSCT arises from the peritransplant morbidity/mortality. However recent transplant procedures especially in young patients unexposed to years of transfusions and other therapies make HSCT an attractive cure with rapid immune recovery.

**P987**

**DLI following allogeneic CD34+ selected PBPC transplantation in paediatric patients with haematologic malignancies using HLA-identical related donors**

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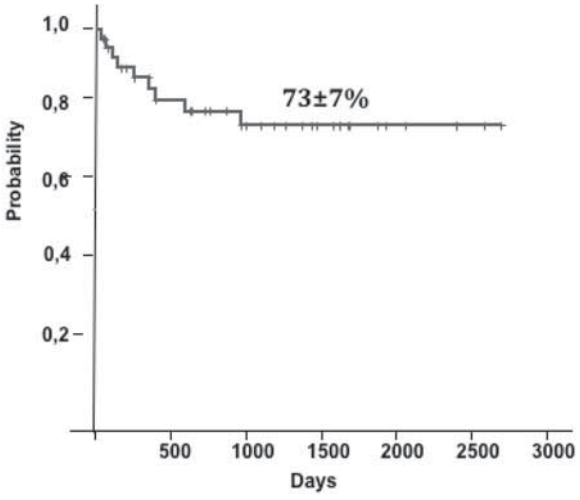
Introduction: Allogeneic CD34+ selected peripheral blood progenitor cell (PBPC) transplantation results on fast engraftment with no significant graft-versus-host disease (GvHD). Cellular therapy with donor lymphocyte infusion (DLI) following transplant allows to improve immune reconstitution and enhances graft-versus-leukemia (GvL) effect.
Materials and Methods: We present our results of 43 transplants performed in children with hematologic malignancies from 2004 to 2011. Main diagnosis was: ALL (n=19), AML (n=16), CML (n=3), MDS (n=1) and NHL (n=4). They were transplanted using a fludarabine-RIC and grafted with CD34+ selected PBPC of an HLA-identical (n=39) or one mismatched related donor (n=4). Median age was 8 years (range, 1-16 years). There were 25 male and 18 female. Disease status was 1st CR in 26, 2nd CR in 13 and >2nd CR in 4. It was the 2nd transplant for 2 patients. All conditioning regimens included fludarabine combined with melphalan from 2004 to 2005 (n=12) and with iv busulphan and thiotepa since 2006 to 2011 (n=31). Graft-versus-host disease prophylaxis consisted on cyclosporine ± methotrexate. A median of 2 DLI (range, 0-8) with $1x10^6$ CD3+/Kg (range, 0-158) were infused after transplant.
Results: With a median follow-up of 3 years, disease-free-survival (DFS) was 73±7% (Figure 1). Relapse incidence was 27% and transplant-related-mortality was 5%. Acute and chronic GvHD incidence was 17% and 35%, respectively. Ratio CD4+/CD8+ normalized 6 months after transplant. In multivariate analysis of DFS the prognostic factors were conditioning (86±7% with fludarabine-busulfan-thiotepa and 46±15% with fludarabine-melphalan, p<0.005) and age (93±6% in children and 54±11% in infants and adolescents, p<0.005).
Conclusion: The combination of RIC and transplantation of CD34+ selected grafts resulted on fast engraftment and immunological recovery avoiding severe GvHD preserving the GvL effect.




**[P986]**

| Patients | Age | Mutations | Ferritin µg/lt | Defects | Treatment before HSCT |
|---|---|---|---|---|---|
| Patient 1 | 1yr | rps5 | 498 | Fallot tetralogy | Transfusions |
| Patient 2 | 7yr | rps19 | 8000 | GI malformation Hypoplastic aortic arch pulmonary artery stenosis | Transfusions, metoclopramide, chelation |

**P988**

**Results of treatment of Ph positive acute lymphoblastic leukaemia in Poland: impact of haematopoetic stem cell transplantation and adding tyrosine kinase inhibitors**

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Aim: In this retrospective analysis we aimed to evaluate the role of allogeneic hematopoietic stem cell transplantation (allo-HSCT) tyrosine kinase inhibitors in therapy of childhood Ph+ALL.

Patients and Methods: Between 2000 and 2010 in 14 Polish centers 1607 patients were diagnosed with Acute Lymphoblastic Leukemia (ALL). Based on cytogenetic or molecular study 64 patients (4%) were found to have Philadelphia positive ALL (Ph+ALL).There were 36 (56%) males and 28 (44%) females, median age of these patients was 8 years and 2 months. Patients with Ph+ALL were classified as high risk group and their treatment included induction with prednisone, vincristin, doxorubicin and asparaginase (phase 1) followed by cyclophosphamide, ARA-C and merkaptopurin Patients with identified match related donor were offered allo-HSCT after third course of consolidation therapy.

Results: Induction failure occured in 3 patients (5%), two of them, however, responded to treatment and achieved late remission and finally underwent HSCT. Seven (10%) patients died in remission from treatment related complications during induction and consolidation. Thirty one patients (31/56) underwent allo-HSCT in first CR. Thirteen children were treated with imatinib along with induction and/or consolidation therapy. Decision to add imatinib to treatment protocol was made on individual basis by the local physician and twelve of these patients underwent allo-HSCT. Overall survival (OS) of the Ph+ALL patients in our group was 0,69 at 3-years and 0,55 at 5 years follow-up. There were no differences in OS between transplanted and not transplanted patients: 3-year OS was 0,71 and 0,68 and 5-years OS was 0,52 and 0,58 subsequently. No difference in OS was found also between patients treated and not with imatinib: 3-years OS was 0,66 and 0,68 subsequently. In group treated with imatinib we noted significantly lower relapse rate (1/13, 8%) as compare to remaining patients achieving first CR (14/43, 27%) however in this group higher number of treatment related deaths occurred after HSCT 5/13 (38%) as compare to patients treated without imatinib 4/43 (9%).

Conclusion: We concluded that alloHSCT in first CR may not improve outcome of pediatric Ph+ALL patients. Adding imatinib to treatment protocol could be associated with higher treatment related mortality but seems to improve remission rate in this patients. Further study are necessary for optimizing treatment of Ph+ALL pediatric patients.

**P989**

**ATG-F can substitute OKT3 in conditioning regimens for haploidentical T- and B-cell depleted stem cell transplantation in paediatric patients**

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T and B cell depletion of haploidentical peripheral stem cells with CD3/19 coated magnetic microbeads prevents GvHD and allows to coinfuse large numbers of donor NK cells. The anti CD3 specific OKT3 antibody was routinely used as rejection prophylaxis without affecting CD56+ NK cells. However, due to its restricted availability, the substance has to be substituted by polyclonal ATG preparations comprising also the CD56 antigen. We present data with reduced ATG doses given at start of the conditioning regimen in order not to impair cotransfused NK cells. A total of 15 patients (ALL n=4, relapsed solid tumors n=9, SAA, SCID n=2) received either 3x5 mg/kg (n=7) or 3x10 mg/kg (n=8) ATG-F (Fresenius) starting at day -12, followed by fludarabine (160mg/m$^2$, day -8 to -5) thiotepa (10 mg/m$^2$ day -4) and melphalan (140 mg/m$^2$ day -3 to -2). A median number of 13,8x106 CD34/kg and 64x106 NK cells/kg with 16x103 T cells/kg was infused. Median time to ANC>500 was 9 days in both groups. Graft rejection occurred in 3/7 patients with 15 mgATG (42%) and in 0/8 patients with 30 mg ATG. After reconditioning, final engraftment was achieved in all patients. Acute GvHD grade II-IV was observed in 1/7 (15 mg) and 0/8 (30 mg) patients. Chronic GvHD occurred in 1 patient (15 mg group). Immune recovery of CD56+ NK cells was fast with a mean number of 461 *vs* 413 cells/µl at day +14, 448 *vs* 294 cells/µl at day +30 and 206 *vs* 147 cells/µl at day +90. CD3+ T cells reached 109 *vs* 16/µl at day +30 and 120 *vs* 68/µl at day +90 (15 *vs* 30 mg group). ATG serum levels were measured in 8 patients with flow cytometry (amount of ATG binding to the Jurkat cell line, defined as specific rabbit IgG). Median peak levels of 11.4 µg/ml (15 mg group) and 15.8 µg/ml (30 mg group) specific rabbit IgG were reached between day -8 and -6 and dropped to 1.2 and 3.1 µg/ml at day 0. Conclusions: Our aim was to substitute OKT3 by ATG in patients who receive CD3/19 depleted haploidentical peripheral stem cells without hampering immune recovery and donor NK cells infused on day 0. Administration of 15 or 30 mg/kg ATG-F was started at an early time point (day -12) of the regimen. Both doses resulted in low serum levels of specific ATG at day 0 and in a fast NK cell recovery. T cells recovered slightly faster after 15 mg/kg than after 30 mg/kg of ATG. However, 15 mg/kg seemed to be less effective in preventing graft rejection. Thus, we recommend to use 30 mg/kg of ATG-F in this setting. Further investigations are necesssary to confirm these data.

**P990**

**Acute GvHD in paediatric recipients of haematopoietic stem cell transplantation. A single-centre experience over the last decade**

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Introduction: Children are generally considered at reduced risk of severe acute Graft versus Host Disease (aGVHD), even

though its probability increases when alternative donors (AD) are used. We analyzed HSCTs performed in our center over the last decade, with the aim to evaluate risk factors for aGVHD, scored according to Glucksberg and NIH criteria, and its influence on transplant-related mortality (TRM) and malignant recurrence.
Patients and Methods: Data were analyzed as of June 11'. Recipients of 2nd or subsequent HSCT and of *in vitro* T-cell depleted graft were excluded from analysis.Recipient gender and age, diagnosis(malignant *vs* non-malignant), donor type (match-related donor-MRD *vs* AD), stem cells source (BM *vs* PBSC *vs* UCB), type of preparative regimen (myeloablative *vs* non-myeloablative: MA *vs* nMA) were considered as potential risk factors.
Results: 197 pts (median age 8.5 yrs: range 0.3-18) were eligible for the study; 66% of HSCTs were performed for malignant diseases and AD was used in 72% of cases. BM was used in 81% (PBSC:6%; UCB:13%),whereas the majority of pts (76%) received MA regimen. Children who failed to engraft (6%) or died/relapsed before engraftment (3%) were excluded from the analysis, thus allowing 179 evaluable pts. AGVHD occurred after 134/179 (75%) HSCTs and classified as follow: grades 0-I:48%; II-IV:52%; III-IV24%; classic 69%; persistent/recurrent/late onset:31%. In univariate analysis, none of the evaluated factors influenced the risk of developing severe Glucksberg grades of aGvHD; when evaluated according to NIH criteria, pts with malignancies and those receiving MA regimens resulted at increased risk for classic form of aGvHD ($p<0.01$). After a median follow-up of 3,4 yrs (range 0,07-11,3), probabilities of overall survival (OS) and TRM were 72% (CI: 64-78) and 15% (10-21), significantly influenced by the degree of aGVHD (0-I:81% and 2%; II-IV:63% and 26%; $p<0.05$). In malignant disorders, more severe aGVHD correlated with a reduced risk of malignant recurrence (II-IV:19%; 0-I:28%) but also with increased TRM (II-IV:21%; 0-I:3%; $p<0.01$). In non-malignant disorders, degree of aGVHD significantly influenced TRM and OS ($p<0.01$).
Conclusions: In our experience, the probability of severe aGvHD was similar regardless of type of donor and source of stem cells, while children with malignancies and use of MA correlated with an increased risk for classic aGvHD. Children with non malignant diseases are best candidates to benefit of novel approaches to prevent aGVHD.

**P991**

**Single-centre experience in the safe administration of mesenchymal stem cells in paediatric patients**

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Introduction: The use of mesenchymal stem cells (MSC) in the subset of paediatric patients is particularly scanty. In the literature, the MSC administered in these patients have been expanded using fetal calf serum, however in our institution we have implemented the expansion protocol from bone marrow derived MSC under GMP conditions using platelet lysate without any other supplementary factor in the culture medium and cryopreserving cells in AB plasma with 10% of DMSO.
In the general practice, MSC are administered in our institution for adults that develop a complication of the allogeneic transplant of haematopoietic cells, such as graft versus host disease (GVHD) or peripheral cytopenias. The use of cells is regulated by either clinical trials or compassionate use. However in children there is no clinical trial available, so there are no paediatric doses of MSC usually available.
Objective: The aim of this work is to describe our experience using clinical grade bone marrow derived MSC (BM-MSC) in children, expanded using platelet lysate, cryopreserved and infusing them after thawing.
Patients and Methods: Four children have been treated with BM-MSC. All of them had received a matched unrelated donor for the following diseases: major thalasemia, juvenile myelomonocytic leukemia, haemophagocytic syndrome and Hurler syndrome respectively.

**[P991]**

Table 1: Characteristics of the patients, and results of BM-MSC administration

| Patient | Age (years) | Disease | Transplant complication | Day postransplant | Dose of MSC per infusion | Number of infusions | Results |
|---|---|---|---|---|---|---|---|
| 1 | 3 | Major thalasemia | aGVHD | +95 | $3x10^6$/kg | 10 | Partial response |
| 2 | 11 | Juvenile myelomonocytic leukemia | aGVHD | +28 | $1.7x10^6$/kg | 4 | Gut: Complete response Liver: No response |
| 3 | 1 | Haemophagocytic syndrome | Graft failure | +120 | $5.4x10^6$/kg | 2 | Complete response |
| 4 | 2 | Hurler syndrome | ITP | +180 | $3.7x10^6$/kg | 4 | Complete response |

The first patient developed severe acute gut GVHD refractory to two previous lines of treatment. The second one suffered gut and liver acute GVHD refractory to steroids. The other two were diagnosed of graft failure and immune thrombocytopenia (ITP).
In all cases the use of MSC was approved by compassionate program use, and was considered urgent due to the clinical status of the patients, so third party donor cryopreserved cells, available in our institution were administered.
MSC were thawed and washed with saline solution with 5% of human albumin, resuspended and counted. Doses between 1.7 and 5.4 $x10^6$/Kg were iv administered. Dexclorfeniramine and steroids were previously used in order to prevent any unexpected reaction.
According to response patients received between 2 and 10 infusions. In no case any adverse event was reported. Responses are summarized in Table 1.
Conclusions: This protocol is safe for children, on one hand washing cells in children with less than 20kg and on the other in elderly children directly infusing in plasma and DMSO. The exact age/weight in which washing is no longer necessary remains to be determined.

**P992**

**Cyclosporine-induced dyslipidaemia in a paediatric stem cell transplant patient**

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Calcineurine inhibitors have played a major role in the immunosuppressive regimen in the stem cell transplant setting since their introduction in 1980. Cyclosporine and Tacrolimus are the two drugs in this class and are used in most transplant settings with out a major difference in their efficacy.
Both drugs act via the T cell activator protein calcineurine, inhibiting T cell activation and cytokine gene expression. Dyslipidemia occurs in 45% to 80% of solid organ transplantation patients on immunosuppressive therapy. Tacrolimus shows less frequent and milder effect on the lipid profile compared to cyclosporine. There are no documented reports of dyslipidemia in any pediatric stem cell transplant patients to our knowledge.
We report a case of Cyclosporine induced dyslipidemia in an 11 year old male patient. The patient received a matched sibling bone marrow following a preparative regimen of Cyclophosphamide, Fludarabine and ATG to treat his idiopathic severe aplastic anemia. He received methotrexate day +1, +3 and +6 along with Cyclosporine for GVHD prophylaxis with the plan to continue Cyclosporine for at least 9 months due to his underlying disease. He had a family history of dyslipidemia with father on lipid lowering agents. His weight and BMI at the start of conditioning were 69.4 kg and 28.5 respectively. His pre-transplant full lipid profile is unavailable but his serum triglyceride was normal at 150 mg/dl. At the time of engraftment he received 3 days of pulsed steroids for engraftment syndrome.
During the first month post transplant, his blood was persistently lipemic, creating difficulty obtaining accurate chemistry panels. He failed a conservative approach to control his altered lipid levels with maximum triglyceride reaching 1117 mg/dl. At 5 months following transplant, fibric acid derivative was added and his cyclosporine was changed to Tacrolimus. His lipid profile started trending down 4 weeks in to this change and is in the range of 200 mg/dl at this stage. He is on a tapering schedule of Tacrolimus and will be off in 1 month. Dyslipidemia can occur with calcineurine inhibitors in the pediatric stem cell transplant setting in high risk individuals. Monitoring for this potential complication is warranted in high risk individuals. Tacrolimus may have an advantage over Cyclosporine in this setting.

**P993**

**A retrospective analysis of risk factors, results of pre-emptive therapy and outcome of CMV infection in paediatric patients who underwent allogeneic stem cell transplantation**

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CMV infection is the most frequent viral complication in the early post-transplant period. In adults, the pre-emptive use of ganciclovir and foscarnet resulted comparable as efficacy, the toxicity profile being slightly different. Limited data are available on the safety and efficacy of foscarnet in pediatric patients.
We analyze retrospectively the risk factors, results of treatment, and outcome of CMV infection in 177 pediatric patients who had undergone a first allogeneic HSCT between April 1997 and March 2009. CMV reactivation was defined by any positivity of pp65 early antigen on leukocytes or a DNAmia > 300 genomic copies/105 mononucleated cells.
The incidence of CMV reactivation was 40% (70/177) at a median of 38 days (range: 3-118 days) after HSCT. In univariate analysis, CMV reactivation was associated to unrelated donor source of stem cells, conditioning regimen containing ATG, and GVHD prophylaxis with CSA-MTX ($p<0.015$). In multivariate analysis, the only factor that remained significant for CMV reactivation was the prophylaxis with CSA-MTX whilst recipient/donor CMV seronegativity resulted protective for CMV reactivation ($p<0.0018$).
Most of the episodes (52/70, 74%) were treated pre-emptively with foscarnet alone or in combination with ganciclovir (7/70, 10%). The remaining episodes (18/70, 26%) were treated only with ganciclovir. Overall, pre-emptive treatment with foscarnet or ganciclovir was well-tolerated. According to WHO scale, grade II and III renal toxicity was recorded in 8 and 2 patients, respectively, both treated with foscarnet. A complete resolution of CMV infection was obtained in 58/70 episodes (83%) whereas in 14 of 70 episodes (20%) there was a progression to CMV disease, i.e. 9 had interstitial pneumonia (1 patient had also pancreatitis and retinitis) and 5 patients had fever. Three of the 14 patients with CMV disease developed acute respiratory distress syndrome and died (21%). Importantly, no difference in 1-year OS was found between patients with or without CMV reactivation/infection: 71% *vs* 74% respectively, $p = 0.69$, I.C:95%.
In conclusion, strict monitoring and pre-emptive treatment are a successful strategy also in pediatric patients. Despite the retrospective nature of the study, we provide evidence that foscarnet is a valid option to ganciclovir also in pediatric patients as first-line treatment of CMV infection, renal toxicity being mild.

**P994**

**A non-inferiority study comparing efficacy and safety of a single dose of pegfilgrastim versus daily filgrastim in paediatric patients after autologous peripheral blood stem cell transplant**

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Objectives: To assess the efficacy of pegfilgrastim *vs.* daily filgrastim in pediatric patients who underwent peripheral blood stem cell transplant (PBSCT) by a randomized, multicenter, phase III, non inferiority study.

Methods: The primary endpoint was the time to polimorphonuclear cell (PMN) engraftment. Patient sample size was calculated on the hyphotesis that a single dose of pegfilgrastim of 100 ug/kg was not inferior to 9 or more doses of filgrastim of 5 ug/kg/day as time of PMN recovery. Platelet (PLT) engraftment, iatrogenic toxicity, and safety were secondary endpoints.

Results: From May 2007 to June 2011, 61 patients (pts), 38 M, 23 F, were recruited in 4 centres: 29 pts were randomized to filgrastim arm and 32 pts to the pegfilgrastim arm.

Median age at PBSC transplantation was 11.5, range 1.6-17.4; median body weight was 36, range 9.6-106; 20% of patients were affected by lymphoma/leukaemia, 80% by solid tumours; 79% of patients were in complete remission (32) or in very good partial remission (16) at PBSC infusion. The median value of CD34+ infused was 6.4x $10^6$/kg, range 3–300.

The mean time to PMN engraftment was 10.48 days (standard deviation (SD) 1.57) and 10.44 days (SD 2.44) in filgrastim and pegfilgrastim group, respectively. Having fixed a non inferiority margin Delta = 3, the primary endpoint was reached, determining the non inferiority of pegfilgrastim. Other enpoints in the filgrastim and pegfilgrastim group were as follows: PLT engraftment, 100% *vs.* 97%; median time to recovery to PLT > 50 x $10^9$/L, 22 days, range 10-84 *vs.* 28 days, range 10-132; one or two episodes of FUO, 79% *vs.* 78%; proven infection, 34% *vs.*28%; WHO grade II-IV mucositis, 76% *vs.* 59%. No significant differences were found in terms of toxicity between 2 arms and no toxic death was reported within the first 100 days post-PBSCT. After a median follow-up of 2.3 years (95% C.I.: 1.5-3.3), 20 deaths were observed, 9 in the filgrastim and 11 in the pegfilgrastim group, all due to progression of disease.

Conclusion: A single dose of pegfilgrastim is not inferior to daily filgrastim in pediatric patients who underwent PBSCT.

**P995**

**Pneumatosis intestinalis with CMV infection in a paediatric severe aplastic anaemia patient suffered from graft-versus-host disease after haematopoietic stem cell transplantation**

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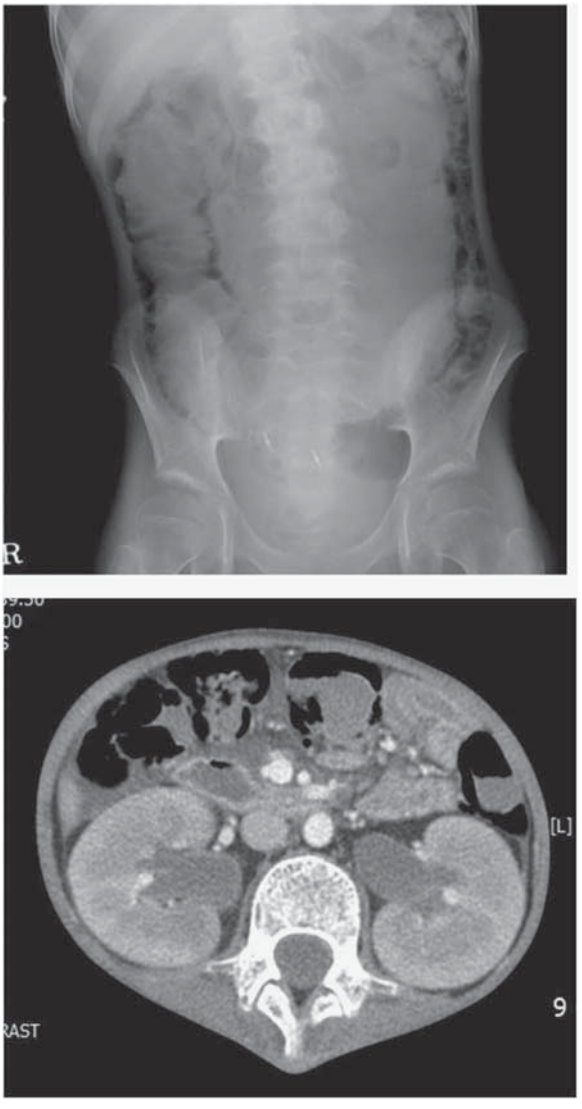
Introduction: Pneumatosis intestinalis (PI) has been reported usually in neonatal period, but may have been seen in immunocompromized patients such as patients after organ transplantation at older children age. The etiology of PI is thought to be mechanical cause or bacterial infection, but has not been known yet.

Case: A 10 year old female girl diagnosed as severe aplastic anemia, who has CMV (Cytomegalovirus) seropositive. She was transplanted with peripheral stem cell from a matched (6/6 high resolution human leukocyte antigens) unrelated donor who has CMV seropositive. The conditioning regimens used at transplantation were Busulfan, Fludarabine, ATG (anti-thymocyte globulin). She was neutrophil engrafted at D (day) +12 after transplantion. GVHD (graft versus host disease) was developed from D+15 and Grade III GI (gastro-intestinal) GVHD and has been controlled with methyl-prednisolone and tacrolimus. The symptom of GI GVHD was wax and wane. At D+114 abdominal pain and distention was developed and at D+115 she got mild fever and watery diarrhea with blood tinged stool. We treated with methyl-prednisolone 1.5 mg/kg and tacrolimus 0.003 mg/kg intravenous injection.

At D+116, PI was detected on simple abdominal X-ray and CT (computed tomography), Conservative management such as



NPO (nothing per oral) and transfusion was maintained. Vital sign was stable, distended abdomen but tenderness was not severe at physical examination. CRP (C-reactive protein) and ESR (erythrocyte sediment ratio) was not elevated. Ganciclovir (5 mg/kg twice a day) was injected intravenously because CMV antigenemia was detected at D+117. AT D+119, PI sign was disappeared on simple abdominal X-ray, and hematochezia decreased gradually. No bacteria was detected at Stool culture, and clostridium difficile toxin A test was negative, CMV antigenemia was disappeared by the day after using of ganciclovir for 10 days.
Conclusion: We describe a 10 year old female who developed PI with CMV infection suffered from gastro-intestinal graft versus host disease (GVHD) after allogeneic stem cell transplantation due to severe aplastic anemia and was treated with ganciclovir successfully.

**P996**

**Lung function after allogeneic stem cell transplantation in a population-based Danish paediatric cohort**

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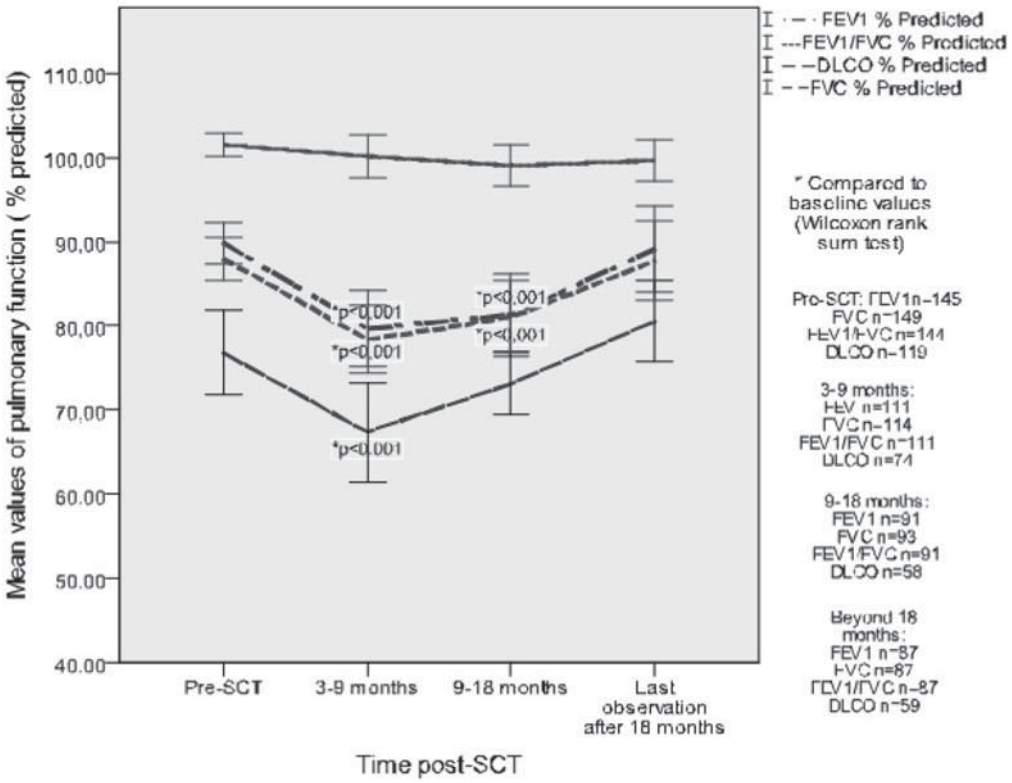
Pulmonary complications are associated with significant morbidity and mortality following stem cell transplantation (SCT), but reported incidence and severity is highly variable. This retrospective study describes lung function in a national paediatric cohort that underwent regular monitoring starting one month pre-SCT.
Methods: Children aged 6-16 years at SCT from 1990 to 2010 (n=180) were included. Lung function tests performed pre-SCT and subsequently 3-9, 9-18 and beyond 18 months post-SCT were collected, and included forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), FEV1/FVC and carbon monoxide diffusion capacity (DLCO), all presented as percent predicted.
Results: Median age at inclusion was 9.8 years (range 6-15.8). At 3 months post-SCT a significant decrease in FEV1 (mean 89.1 *vs.* 79.2%), FVC (87.5 *vs.* 78.3%) and DLCO (77.1 *vs.* 67.4%) (Wilcoxon paired sum test p<0.001) was observed compared to baseline. This decline persisted after 9-18 months in FEV1 (82.4%) and FVC (81.7%) (p< 0.001). However, at last assessment beyond 18 months, lung function did not differ significantly from pre-SCT levels.
The proportion of patients with FEV1 <80% at 3-9 months was higher among patients with acute Graft-versus-Host Disease (aGvHD n=114) (52.9 *vs.* 32.6%, p=0.05) and CMV mismatch (64.5 *vs.* 38.2%, p=0.018) (Fisher exact test), a difference that persisted beyond 18 months (GvHD; 49.9 *vs.* 19.2%, p=0.035, CMV mismatch; 50 *vs.* 25%, p=0.038). Eighteen months post-SCT, aGvHD patients had a reduced FEV1 compared to baseline (mean 84.7 *vs.* 91.5%, p=0.025).

**[P996]**

Figure 1: Development in lung function over time from SCT



HLA-mismatch was more common among patients with reduced DLCO (<80%) at 3-9 months (91.9 *vs.* 67.7%, p=0.015) and 9-18 months (82.8 *vs.*47.8% p=0.016). Older age at SCT was associated with DLCO reduction at 9-18 months (10 *vs.* 12.6 years, p=0.004) and beyond 18 months (10 *vs.* 12.6 years, p=0.002). Variables without association to lung function included sex mismatch, source of stem cells, diagnosis, conditioning with total body irradiation or busulfan p.o (n=26) or i.v. (n=36), Karnofsky score and donor/recipient CMV-status.
Conclusions: Marked transient reduction in FEV1, FVC and DLCO appears to affect the majority of children undergoing SCT. FEV1 reduction appears to be more common and persistent in patients with aGvHD and in CMV-mismatched transplants, while DLCO reduction is associated with HLA-mismatch. Interestingly, we found no association between decreased lung function and busulfan treatment.

**P997**
**25-hydroxyvitamin D deficiency and bone mineral density alterations in paediatric patients who underwent haematopoietic stem cell transplantation**
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Objectives: Vitamin D (vD) has a crucial role in maintaining calcium homeostasis and normal bone structure. Several studies reported vD deficiency as a long-term effect of haematopoietic stem cell transplantation (HSCT) because of reduction in sun exposure, lack of vD in diet, steroid therapy and altered gastrointestinal absorption consequent to intestinal GVHD after HSCT. Little is known about early modifications of vD levels following HSCT and its correlation with bone mineral density (MBD).
Methods: We performed a retrospective analysis of vD status of 31 paediatric pts (19 male) who underwent 42 HSCT (35 allogeneic, 7 autologous) treated in our Institution for cancer and haematologic diseases between 2006 and 2011. The serum levels of 25-hydroxyvitamin D (25(OH)D) were determined before HSCT and every 3 months for 1 year after HSCT. In addition 6 months after HSCT, BMD modifications were analyzed by Dual-Energy x-Ray Absorptiometry (DXA) that allows a non-invasive assessment of bone mineral content. No pts were receiving vD supplements. Median age at HSCT was 9 (range 5-12). vD status was classified on the basis of 25(OH)D serum level according to value reported by National Health and Nutrition Examination Survey as: Deficiency: <15 ng/ml, Insufficiency: 15-29 ng/ml, Adequate: >30 ng/ml.
Results: Median pre-HSCT 25(OH)D serum concentration was 24.6 ng/ml (range 12-55.1), with 1 pt/31 (3%) showing deficient levels, 23/31 (74%) insufficient and 7 (23%) adequate levels. Median concentration of 25(OH)D post-HSCT was 13.4 (range 6.3-36.4)[P<0.0001]: 21 pts (68%) has deficient serum level, 7 (22%) insufficient and 3 (10%) adequate (Table 1).
BMD evaluated by DXA 6 months after HSCT showed a trend in line with 25(OH)D serum level: Z-score of global BMD was evocative of osteopenia in 15/31 (48%) pts (range -1.1/-2.1) and of osteoporosis in 1/31 (3%) pt (-2.9). Z-score calculated at lumbar vertebrae revealed osteopenia in 4/31 (13%) pts (range -1.1/-2.3) and osteoporosis in 1 pt (-2.6).
Conclusions: This retrospective analysis showed that 25(OH)D serum level decreases markedly and precociously in children treated with HSCT and this is associated with an early reduction of global BMD in nearly 50% of the patients at DXA evaluation. These findings suggest the importance of early monitoring of vD status and BMD in HSCT patients in order to start vD supplementation able to prevent severe vD deficiency and its complications.

**P998**
**Very early onset nonalloimmune haemolytic anaemia associated with warm antibodies in a child following T-cell-depleted, haploidentical peripheral stem cell transplantation**
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Immune-mediated hemolytic anemia (IHA) is a relatively common complication following allogeneic hematopoietic stem cell transplantation (HSCT). The majority of cases are alloimmune, the onset is very early and reflect red blood cell antigen incompatibilities. Non-alloimmune haemolysis with absence of alloantibody and the presence of autoantibodies, reported primarily in patients transplanted with T cell depleted (TCD) grafts and classified as early onset (2-8 months) cold antibody type, late onset (6-18 months) warm antibody type. We report a case of very early onset nonalloimmune hemolytic anemia associated with warm antibodies in a child after TCD, haploidentical HSCT. A 12 years old

**[P997]**

| | Pre-HSCT | Post-HSCT | |
|---|---|---|---|
| **Serum 25(OH)D ng/ml** | 24.6 (12-55.1) | 13.4(6.3-36.4) | *P*<0.0001 |
| **Deficiency <15 ng/ml** | 3% | 68% | *P*<0.0001 |
| **Insufficiency 15-29 ng/ml** | 74% | 22% | *P*=0.0001 |
| **Adequate ≥ 30 ng/ml** | 23% | 10% | *P*=0.3 |

**Table1.**
Following HSCT the median concentration of 25(OH)D was significantly lower compared with pre-HSCT levels, with a marked increase of patients with deficient and insufficient serum levels of 25(OH)D.

boy diagnosed as MDS(RAEB-t) underwent 2-loci mismatched haploidentical HSCT from his ABO/Rh/ minor red blood cell antigen matched mother. Immunomagnetic separation method was used for CD34+ selection and T cell-depletion The number of CD34+ and CD3+ cells were $5.58x10^6$/kg and $1.9x10^4$/kg, respectively. Full chimerism and hematopoietic engraftment was achieved on the +18 day. He was in good health condition untill the +28 day when he had presented with clinically evident haemolysis. The serological findings were typical of conventional, idiopathic non-alloimmune hemolytic anemia with warm-reactive, nonspecific antibodies detected. He responded to immunoglobulin and prednisolone with minor evident haemolysis. But he hasn't subsequently done well and progressed to pancytopaenia with further haemolysis after 30 days from the first haemolysis, despite full chimerism. Bone marrow biopsy at day +76 revealed aplastic anemia. He failed to respond supportive care and donor leucocyte infusion (DLI) and died from pulmonary hemorrhage. To our knowledge, this is the first reported case of a very early onset nonalloimmune warm antibodies associated hemolytic anemia. The time course of hemolysis and full chimerism of the recipient in the case suggest that the antibody was produced by donor-derived lymphocytes. Warm type autoantibodies associated with non-alloimmune haemolysis are relatively difficult to treat. Considering the very poor prognosis of TCD, haplo-identical HSCT patients who develop this complication it is very important to choose and perform the most appropriate treatment for the patient immediately.

**P999**

**The result of steroid-resistant GvHD treatment by mesenchymal stem cells infusion in paediatric patients**

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Aim: We analyzed the clinical effect of MSC infusion for treatment of severe steroid-resistant GVHD in children after allogenic HSCT and possibility to withdrawal immunosuppressive therapy in these patients.
Patients and Methods: The 33 children have been included in our study. All patients received GVHD prophylaxis after allogenic hematopoietic stem cell transplantation (HSCT): for pts with ALL / MDS/ CML/ CMML/ NHL/AF consist of CSA and MTX; for pts with SAA - CSA+MMF; for pts with AML - CSA and MTX, for pts with AF methylprednisolone + tacrolimus. For the treatment of GVHD all pts received methylprednisolone 1-2 mg/kg, however developed steroid-resistant acute (grade II-IV) and chronic extensive GVHD. Patients were divided into two groups: 1 group - 22 patients (median age was 10,6 years, male/ female: 18/4) with AML-5, MDS-1, NHL-1, ALL-9, SAA/AF-6 were underwent MSCs infusions after HSCT for treatment of steroid-resistant acute or chronic GVHD. Ten pts received MSCs once and twelve – twice. The median first dose of MSCs was 1,7 (0.5–5.4)$x10^6$/kg and second dose – 1,0 (0.7–2.5)$x10^6$/kg. The MSCs were derived from bone marrow of HSCs donors (HLA-identical donors) (n = 10) and third-party HLA-mismatched donors (n = 12). 2 group - 11 children, (median age was 15 years, male/ female: 5/6) with AML-2, ALL-4, SAA/AF-3, HML-1, HMML-1, who weren't received MSC presented as the control.
Results: No patients had side-effects during or immediately after the infusions of MSCs. The incidence of death from GVHD amounted to 9,1% in group of patients given MSCs (2 pts died from GVHD and sepsis) compare with 55% in group of children which received immunosuppressive therapy alone (6 pts died from GVHD) (p=0,02). Median duration of survival from allogenic HSCT was 33 (6–58) months in group of patients with MSCs and 5 (2–65) months in group pts without MSC (p=0,02). Immunosuppressive drug were withdrawal in 10 pts from 22 children which have got MSC and in 1 pts without MSCs.
Conclusion: This study confirm rational of MSC administration for treatment of steroid-resistant GVHD for reduction death from DVHD and increase of duration of survival from allogenic HSCT in children. Our date suggest that MSCs infusion allows completely withdrawal immunosuppressive therapy in most patients of steroid-resistant GVHD.

**P1000**

**Quality of life in a paediatric population after stem cell or bone marrow transplantation: first qualitative data of a longitudinal follow-up**

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Background: The focus of care for transplanted patients has partially been switched to Quality of Life(QoL) within this population. The long term impact of hematopoietic transplantation is more documented in adults. Pediatric patients are a growing point of interest.
A multidisciplinary follow-up started in september 2010 to evaluate QoL.
We wanted to adress the degree of physical and psychosocial rehabilitation. Additional to logg the data resulting in psychological distress, social dysfunctioning and underachievement at school or work. And finally to get visibility on the individual sympton burden.
Methods: The objective was to collect relevant data and to improve patient care. 36 patients(pre-school to young adults) were seen on the outpatient consultation. The time since transplant ranged between 1 and 17 years, with most of them between 2 and 5 years (16 patients) and between 5 and 10 years (15 patients). Initially we wanted to collect both, quantitative and qualitative data. The PedsQL (4.0 Generic core scales and the PedsQL 3.0 cancer module) generally used to evaluate QoL during active treatment were not suitable for a long term follow up. Although we have a better instrument for this purpose, it could yet not be used as it has to be adapted for our Dutch-speaking population. A semi-structured interview to question the different life domains was done by the transplantnurse and psychologist parallel to the medical consultation.
Results: In general the QoL is relatively good, but with a variety of residual difficulties in a number of patients. These are situated in the area of less endurance and more fatigue, lack of energy, sleep problems, nightmares and flash-backs and problems with physical appearance. Emotionally they report feelings of loneliness, sadness and anger, worrier thoughts, feeling otherwise than peers and sometimes difficulties in social interaction.
On the cognitive level we registered learning problems and a lower capacity in terms of attention and concentration with a potential impact on the choice of study and type of work.
Conclusions and Future Objectives: A multidisciplinary long term follow-up is essential for both research purposes as a better patient care. Our aims are to continue with baseline and regular measurements for QoL before and after transplantation. Additional, collecting quantitative data with an appropriate instrument. And eventually the drafting of curative and preventive protocols.

**P1001**

**Variable clinical presentation and HSCT outcome in patients with infantile osteopetrosis among two ethnic groups**

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*(1)Center for Pediatric Hematology, Oncology, Immunology (Moscow, RU); (2)R.M. Gorbacheva Center for Pediatric Hematology and Transplantology (St. Petersburg, RU); (3)Republican Children's Clinical Hospital (Cheboksary, RU); (4)Hadassah Hebrew University Medical Center (Jerusalem, IL)*

Background: Osteopetrosis (OP) is a descriptive term that refers to a group of rare, heritable disorders of the skeleton characterized by increased bone density results from abnormalities in osteoclast differentiation or function. Recently it was shown an unusually high prevalence of OP in the Middle Volga basin (Chuvashia, Russia) and an unique splice site mutation c.807+5G>A at the TCIRG1 in 11 Chuvashian patients (Bliznetz *et al*., 2009). Until recently, HSCT was not used to treat OP patients in Russia.

Aims: (1) comparing OP phenotype of Middle Volga basin and Middle East areas; (2) evaluating the role of the conditioning protocol in HSCT outcome.

Methods: We retrospectively evaluated clinical presentation of OP in 43 patients: 17 from Russia and 26 from Israel.

Results: While the age at diagnosis was almost the same (average 6.5 *vs* 8 mo), the structure of symptoms was statistically significant, p=0.004 (Figure 1). Despite high prevalence of at least one neurological symptom at the median age in this group was 40 mo (range from 5 to 86 mo) without transplantation 10/17 patients have relatively mild disease course and still have no intellectual damage and therefore can be good candidates for BMT. One possible explanation of this difference could be different genetic background. In 13/14 Chuvashian children c.807+5G>A mutation was found (8/13 are homozygose); with two patients 4 new TCIRG1 mutations were detected (data not presented). One patient of Ukrainian origin was screened for Chuvashian mutation and found to be negative. Subsequently different mutations in TCIRG1 were found.

Three patients were already transplanted in St. Petersburg (Russia), are alive and well up to 36 months.

Analysis of 26 patients transplanted at Hadassah (Israel) showed significant (p=0.0153) improvement of survival rate with those transplanted 2000-ies *vs* 1983 to 1999 (Figure 2). The introduction of fludarabine-based conditioning protocol in all resent subgroup of the patients makes the major difference between these two groups.

Conclusions: Bone marrow transplantation can be considered for older age OP, especially if the patient is intellectually saved. Screening for c.807+5G>A TCIRG1 mutation is reasonable as the first step approach but not enough for genetic counseling in extended Volga basin population. Experience with different conditioning protocols planned to be used for subsequent PBSC/BMT.

**[P1001]**

**Figure 1:** Symptoms distributions in the Middle Volga Basin vs Middle East patients.

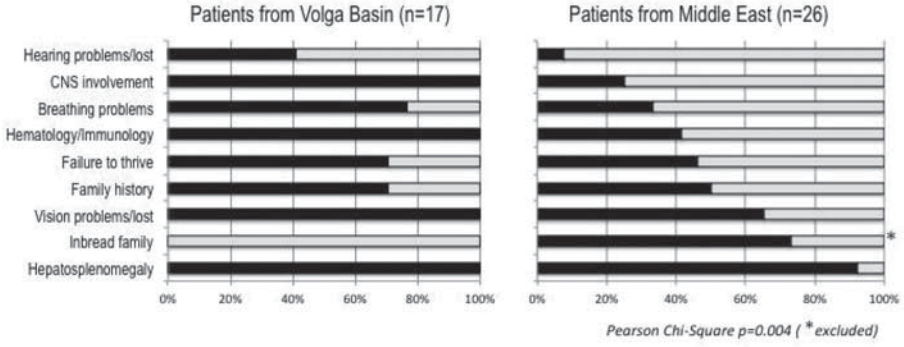
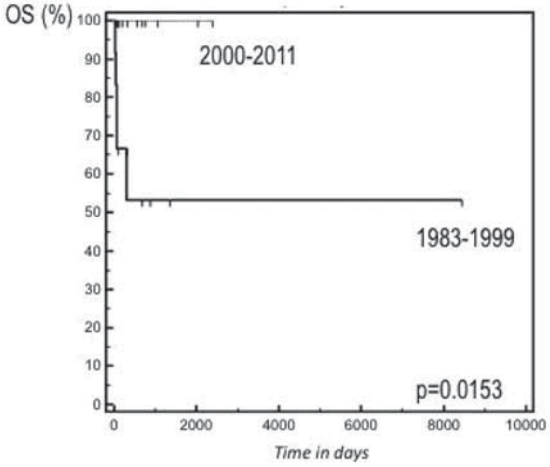



**Figure 2**. A Kaplan-Meyer estimated overall survival of OP patient transplanted before and after the year 1999.



**P1002**

**High-dose chemotherapy supported by autologous stem cell transplantation in patients with Hodgkin's lymphoma. 14 years experience in a paediatric public institution in Argentina**

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Purpose: Evaluate ASCT outcomes in Hodgkin´s Lymphoma in children.
Patients and Methods: From 9/1998-9/2011, we performed 89 ASCT, 26 HL pts (29%), male 18/female 8 (r2,25-1), median age at ASCT was 13,5 years (r7-18y), stage at diagnosis: II 9 pts (34,5%), III 7pts (27%), IV 10 pts (38,5%), Bulky disease 6pts (23%), B symptoms 19 pts (73%). Histology: nodular sclerosis 18 pts (69,3%), mixed cellularity 6 pts (23%), lymphocyte depletion 2 pt (7,7%).
Previous treatments COPP-ABVD 14 pts (54%), AVBD 9 pts (35%), others (11%). 17 pts (65,4%) received additional radiotherapy in involved areas.
Rescued protocols: ESHAP 8 pts (30,7%), IEP/ABVD 7 pts (27%), ICE 4 pts (15,3%), others 7 pts (27 %).
Status at ASCT: 1st CR 2 pts, 2nd CR 17 pts, 3rd CR 2 pts and PR 5 pts.
Results: Median time from diagnosis to relapse was 26.2 months, (r 8-83 m), median time from relapsed to ASCT 10 months (r 4-29 m).
Peripheral blood stem cell (PBSC) was used in all patients, 15 pts received CPM/G-CSF and 11 pts only G-CSF. Median CD34 was $5,85x10^6$ Kg (1,45-17,3) and median TNC was $16,43x 10^8$Kg (2,4-43,1).
Conditioning regimens were CVB in 24 pts (92,3%), others 2 pts (7,7%) ARA-C/Mel/Carmustine 1 pt and Bu/Cy/Mel 1pt
Median time neutrophil engraftment (≥$500/10^9$×L) was 10,9 days (9-33d), and for platelets (≥$20/10^9$×L) 15,5 days (9-33d). The TRM was 0%.
At 120 months the KM probability of overall survival (OS) was 0.66±0.13%, and event free survival (EFS) 0.62±0.13%. for those patients in CR at ASCT, the OS was 0.68±0.14 and the EFS 0.64±013, in PR 0.66±0.27 and 0.67±0.27 respectively, (log rank NS). Cumulative incidence of relapse: 38%.
Conclusions: ASCT in HL in CR or PR is an effective and safe procedure. In our preliminary analysis the status of disease did not impact on the results, but longer follow-up is necessary.

**P1003**

**Seizures in children undergoing haematopoietic stem cell transplantation**

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Objectives: Seizures are a possible manifestation of the neurological complications of hematopoietic stem cell transplantation (HSCT): seizures have been reported in approximately 7% of all transplanted patients and appear to be a poor prognostic indicator. In HSCT setting many factors have been recognized

**[P1003]**

| | Age/Sex | Disease | Conditioning | Type of HSCT | SSC | GvHD prophylaxis | aGvHD (Grade) | Outcome (Years) |
|---|---|---|---|---|---|---|---|---|
| 1 | 3/M | Thalassemia | BU, TT, FLU | Allo | BM | CsA, MTX | No | ANED at 7 y |
| 2 | 11/M | ALL | TT, CY | Allo | BM | CsA | Yes (II) | Died |
| 3 | 1/F | CNS tumor | CY, Carbo | Auto | PBSC | - | - | Died |
| 4 | 10/F | Aplastic Anemia | Flu, CY | Allo | BM | CsA | No | ANED at 1 y |
| 5 | 7/F | NHL | TT, CY | Allo | BM | CsA | Yes (II) | ANED at 9 y |
| 6 | 14/M | ALL | TT, CY | Allo | PBSC | CsA | No | Died |
| 7 | 12/F | AML | BU, TT, CY | Allo | CORD | CsA | Yes (I) | Died |
| 8 | 7/F | AML | BU, CY, L-PAM | Allo | CORD | CsA | No | Died |
| 9 | 7/M | AML | BU, CY, L-PAM | Allo | CORD | CsA, PDN | No | Died |
| 10 | 5/M | Thrombocytopenia | BU, TT, Flu | Allo | BM | CsA, MTX | No | Died |
| 11 | 16/M | AML | BU, CY, L-PAM | Allo | PBSC | CsA | No | ANED at 10 y |
| 12 | 5/M | Drepanocitosis | TT, Treo, Flu | Allo | BM | CsA | Yes (IV) | ANED at 4 y |
| 13 | 10/F | AML | Treo, Flu | Allo | BM | CsA | No | Died |
| 14 | 17/M | ALL | BU, TT, CY | Allo | BM | CsA, MTX | No | Died |
| 15 | 1/F | AML | BU, TT, Flu | Allo | PBSC | - | No | ANED at 6 y |
| 16 | 3/M | AML | Treo, Flu, L-PAM | Allo | CORD | CsA, PDN | Yes (III) | Died |
| 17 | 6/F | ALL | TT, Treo, Flu | Allo | BM | CsA, MTX | No | Died |
| 18 | 6/M | AML | BU, Flu, L-PAM | Allo | BM | CsA, PDN | No | Died |
| 19 | 14/F | CNS tumor | TT, L-PAM | Auto | BM | - | - | Died |
| 20 | 3/M | CNS tumor | TT, L-PAM | Auto | PBSC | - | - | Died |
| 21 | 11/M | RMS | TT, L-PAM | Allo | BM | CsA | Yes (II) | Died |
| 22 | 17/F | Thalassemia | TT, Treo, Flu | Allo | BM | CsA, PDN | No | Died |
| 23 | 11/M | HL | Eto, TT, CY | Auto | BM | CsA | No | ANED at 6 y |
| 24 | 11/M | BDA | TT, Treo, FLu | Allo | BM | CsA, MTX | No | ANED at 4 y |
| 25 | 1/M | HLH | BU, Flu, Thio | Allo | CORD | FK, PDN | No | Died |
| 26 | 10/M | ALL | BU, Thio, CY | Allo | PBSC | CsA, MTX | No | Died |
| 27 | 4/M | Thalassemia | BU, Flu, Thio | Allo | BM | CsA, MTX | No | ANED at 7 y |

Table 1. Clinical characteristic of the patiens are here summarized.

Legend: SSC: source of stem cell. AML= acute myeloid leukaemia; ALL= acute lymphoblastic leukemia; HLH: Hemophagocytic lymphoistiocitosis BDA: Blackfan-Diamond Anemia, RMS: rhabdomyosarcoma BM= bone marrow; PSBC= peripheral stem blood cells; CNS: Central Nervous System; BU= busulphan; Carbo: Carboplatin, L-PAM= melphalan; Flu: Fludarabine, Treo: treosulphan, TT= Thiotepa; CSA= cyclosporine; ETO: etoposide, PDN= methylprednisolone; MTX= methotrexate

to be possibly associated with the onset of seizures (infections, cerebral disease, bleeding, drug toxicity, hypomagnesaemia). Aim of this study is to retrospectively evaluate incidence and features of crises in children tranplanted c/o our center during the last ten years.
Methods: 345 HSCT procedures (180 autologus, 155 allogeneic, 10 haploidentical) in 261 consecutive children tranplanted between 2000 and 2010 have been reviewed. The medical history, transplant variables, EEG and neuroimaging of patients between 0 and 18 years with a definite diagnosis of seizures within one year after transplant were analyzed.
Results: 27/261 (10.34%) patients had seizures, of these, 23 received allogeneic and 4 autologous HSCT. Nine patients (33%) are still alive and well after a follow-up of six years, eighteen patients (67%) are died (Table 1). The etiology of the crisis was: reversible posterior encephalopathy (PRES) in 14, CNS infections in 4, dimethyl sulfoxide toxicity in one case. 4 patients had epilepsy secondary to underlying disease and in other 4 we have not been able to identify the etiology. The crises have raised an average of 100 days after HSCT [-2, +352]. Crises often begin with ocular and visual signs and/or symptoms, particularly in PRES. 14/27 patients had status epilepticus (SE), in 5 cases non-convulsive; 12 of them were cases of PRES. 83% of patients with SE needed for intensive care life support. In children with PRES, both critical and postcritical EEG showed peculiar abnormalities in the electrical activity prevailing on the posterior regions, in others there was not a specific pattern EEG.
Conclusions: Our data confirm that crises are a common and severe complication of HSCT they are a poor prognostic indicator. PRES is the most common cause of crisis. At the onset ocular and visual signs and symptoms are often present. These features tend to be of long duration and they are related to EEG abnormalities on posterior regions. The recognition of this pattern is suggestive, so the use of a close EEG monitoring could be useful for making a correct diagnosis, treatment and management of these patients.

**P1004**

**Prospective serial ultrasound of the liver is a useful method to detect hepatic abnormalities in children with normal liver function after haematopoietic stem cell transplantation**

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Objectives: Hepatic conditions like steatosis, haemangiomas, focal nodular hyperplasia (FNH) and nodular regenerative hyperplasia (NRH) are rare in children but are reported to be common after intensive treatment for pediatric cancer and particularly after hematopoietic stem cell transplantation (HSCT). Many risk factors linked to the HSCT procedure like mediactions, infection, sinusoidal obstructive syndrome, acute and chronic GVHD, and iron overload, have been identified to play a crucial role in the pathogenesis of these diseases. In most of these conditions the biochemical parameters of liver function are normal. Here we report how a prospective serial ultrasound (US) monitoring of the liver can detect hepatic abnormalities in children with normal liver function after HSCT.
Methods: A pre-HSCT liver US and serial liver US at 3 or 6-monthly intervals for a median time of 3 years after HSCT has been performed in 70 children c/o our Institution between 2006 and 2011. Three-monthly assessment of liver function was evaluated by serological determination of transaminases, gamma-GT, alkaline phosphatase, bilirubin, ferritine, albumin and coagulation status.

**[P1004]**

Table 1. Case series data

| case | event | Sex/ Age | Diagnosis | FUP months | Radiotherapy before HSCT | Iron overload before HSCT | Type of HSCT | Donor | Conditioning regimen | SOS | Hepatic aGVHD | Hepatic cGVHD | Viral hepatitis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | FNH | M/6 | ES | 37 | LUNG | NO | AUTO | - | BUS + L-PAM | NO | - | - | NO |
| 2 | FNH | M/8 | ALL | 91 | TBI | NO | ALLO | MUD | EDX + THIOTEPA + TBI | NO | NO | YES | NO |
| 3 | FNH | F/15 | ES | 13 | LUNG + PELVIS | NO | ALLO | MUD | THIOTEPA + LPAM | NO | YES | NO | NO |
| 4 | angioma | M/12 | ALL | 49 | TBI | NO | ALLO | SIBLING | EDX + THIOTEPA + TBI | NO | NO | YES | NO |
| 5 | angioma | F/10 | AML | 60 | NO | NO | ALLO | SIBLING | BUS + THIOTEPA + EDX | NO | NO | NO | NO |
| 6 | fibrosis | M/11 | HD | 24 | MANTLE | NO | ALLO | MUD | FLUDA + EDX | NO | NO | YES | NO |
| 7 | fibrosis | M/2 | NBL | 12 | THORAX | NO | AUTO | - | BU – L-PAM | NO | - | - | NO |
| 8 | fibrosis | M/8 | ALL | 4 | NO | NO | ALLO | MUD | BU + CY +L-PAM | NO | NO | NO | NO |
| 9 | steatosis/ hepatomegaly | M/1 | HLH | 61 | NO | YES | ALLO | MUD | BUS + THIOTEPA + FLUDA | NO | NO | NO | NO |
| 10 | steatosis/ hepatomegaly | M/11 | ES | 3 | LUNG + LEFT ARM + SPINE | NO | ALLO | SIBLING | THIOTEPA + L-PAM | NO | NO | YES | NO |
| 11 | steatosis/ hepatomegaly | M/3 | ALL | 47 | TBI | NO | ALLO | SIBLING | EDX + THIOTEPA + TBI | NO | NO | NO | NO |
| 12 | steatosis/ hepatomegaly | F/3 | AML | 50 | NO | NO | ALLO | MUD | THIOTEPA + TREOSULFANO + FLUDA | NO | NO | NO | NO |
| 13 | steatosis/ hepatomegaly | F/4 | ALL | 30 | NO | NO | ALLO | MUD | BUS + THIOTEPA + EDX | NO | NO | NO | NO |
| 14 | steatosis/ hepatomegaly | M/16 | ES | 19 | PELVIS | NO | AUTO | - | BUS + L-PAM | NO | - | - | NO |
| 15 | steatosis/ hepatomegaly | F/15 | AML | 7 | NO | NO | ALLO | SIBLING | BUS + EDX + L-PAM | YES | NO | NO | NO |
| 16 | steatosis/ hepatomegaly | M/4 | NBL | 4 | LEFT LEG | NO | ALLO | MUD | THIOTEPA + L-PAM | NO | NO | NO | NO |
| 17 | steatosis/ hepatomegaly | F/12 | MEDULLO | 10 | SNC | NO | AUTO | - | THIOTEPA (X3) | NO | - | - | NO |
| 18 | hepatomegaly | M/4 | NHD | 10 | NO | NO | ALLO | MUD | BUS + EDX + L-PAM | NO | NO | NO | NO |
| 19 | hepatomegaly | M/0.2 | HLH | 60 | NO | NO | ALLO | MUD | BUS + THIOTEPA + FLUDA | NO | YES | NO | NO |
| 20 | hepatomegaly | F/6 | HD | 4 | SUPRA-DIAPHRAGMATIC + NECK | NO | AUTO | - | ETO + THIOTEPA + EDX | NO | - | - | NO |

**Table legend:** FUP: Follow-up; HSCT: Hematopoietic Stem Cell Transplantation; SOS: Sinusoidal Obstruction Syndrome; aGVHD: acute Graft Versus Host Disease; cGVHD : chronic Graft Versus Host Disease; FNH : Focal Nodular Hyperplasia; TBI : Total Body Irradiation; MUD: matched unrelated donor; FLUDA: fludarabina; EDX: endoxan; BUS: busulfan; L-PAM: melphalan; Dg: diagnosis; AML: acute myeloid leukaemia; ALL: acute lymphoblastic leukemia; HLH: Hemophagocytic lymphohistiocitosis; NBL: neuroblastoma; ES: ewing sarcoma; HD: hodgkin disease; MEDULLO: medulloblastoma; NHD: non-hodgkin limphoma

Results: A total of 256 hepatic US were performed in 70 transplanted pts (38 AUTO, 32 ALLO) pre and post HSCT. In the pre-HSCT evaluation 6/70 pts presented mild hepatomegaly without steatosis and 1/70 pts presented signs of iron overload. In the post HSCT US evaluation, 4/6 pts with hepatomegaly presented normal liver size after a median FUP time of 8 months while the others persisted with hepatomegaly over 2 years. The signs of iron overload progressively decreased and were no more evident after 2 years. Twenty new diagnoses (see Table 1 for clinical features) of hepatic abnormalities were performed: 3/70 (4.2%) and 9/70 (12.8%) presented hepatomegaly alone and associated to steatosis respectively, while 3/70 (4.2%) had mild hepatic fibrosis. In 2/70 (2.8%) pts a hemangioma not previously detected was diagnosed. Three out of 70 pts (4.2%) developed a FNH at a median FUP time of 30 months. No NRH, cirrosis or malignant neoplasms of the liver were detected. All of the 20/70 (25.5%) cases with hepatic abnormalities were asymptomatic and the liver function was normal.
Conclusions: Serial post-HSCT liver US is a useful tool to facilitate diagnosis of novel hepatic benign conditions in absence of any signs of liver dysfunction. In transplanted children steatosis and FNH appear to be the most common abnormalities.

**P1005**

**Less veno-occlusive disease after intravenous versus oral busulfan for autologous haematopoietic stem cell transplantation: the Belgian paediatric experience**

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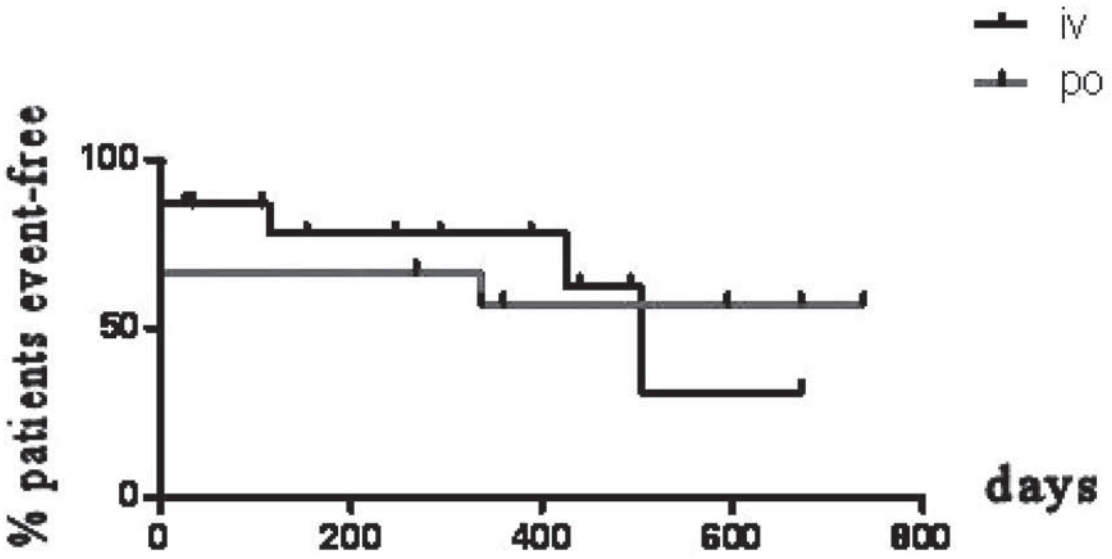
Introduction: Busulfan is commonly used in preparative conditioning regimens prior to hematopoietic stem cell transplantation (HSCT) in children and young adults for malignant and non-malignant disorders. For many years Busulfan was only available in an oral form, resulting in large inter- and intra-patients variability in plasma exposure, associated with higher graft failure rate as well as higher toxicity such as veno-occlusive disease. With the development of an intravenous formulation of Busulfan, a more accurate control of both the inter- and intra-patient variability has been provided.
Objectives: The goal of this study was to evaluate the use and efficacy of intravenous Busulfan (BuIV) in comparison with the oral formulation (BuPO) in children undergoing an autologous transplantation after conditioning with Busulfan.
Methods: Twenty-seven paediatric patients who underwent autologous HSCT between January 2008 and December 2010 in Belgium and received intravenous or oral busulfan as part of their conditioning regimen were retrospectively enrolled in this study. The diagnosis included Ewing Sarcoma, Neuroblastoma and Burkitt Lymphoma. Epidemiologic and transplantation data were collected using the EBMT database (form A).
Results: Bu was administered in all children as part of their conditioning regimens before transplantation. Twelve patients received oral Bu and 15 patients intravenous Bu.
All except 2 patients received standard Bu-Mel regimen followed by autologous HSCT as consolidation therapy. One patient received additional doses of Aracytine and one patient Bu alone. The use of a 5-level dose schedule defined by body weight resulted in an efficient engraftment with marked reduction in the incidence of veno-occlusive disease in the BuIV group compared with BuPO (p=0.028). In terms of disease-free outcome, survival and event-free survival, similar results have been obtained in both groups.
Conclusion: Despite the small number of patients, this study confirmed the apparent benefit of intravenous Busulfan in children undergoing an autologous HSCT. The choice of this formulation of Busulfan should therefore be considered.

**[P1005]**

**Fig 2: Event-free Survival of the BuIV group versus BuPO group**



**P1006**

**Tandem high-dose chemotherapy with thiotepa and Bu-Mel and autologous stem cell transplantation: the way to improve very high-risk neuroblastoma patients prognosis?**

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The administration of Bu-Mel and ASCT has been recently demonstrated to allow the best prognosis for patients with high-risk neuroblastoma (HR-NBL-1 SIOPEN Study). However, patients with less than a partial remission after 2 lines of conventional chemotherapy and adolescents and considered as very high risk (VHR) patients.
We developed an intensified HDC strategy with 2 courses of HDC to improve VHR patients prognosis.
The first course consisted in thiotepa (300 mg/m$^2$/d x3) followed by ASCT. In absence of major toxicity or disease progression, a 2nd course of melphalan (140 mg/m$^2$) Busulphan (600 mg/m$^2$) and ASCT was administered 2 months later. From April 1986 to April 2009, 22 patients (12 males, 10 females), median age 3.5 y (0.9-15.9) entered this programme.
20/22 had less than a partial remission after conventional chemotherapy. 2 were adolescents with a metastatic CR, 1 had a MYCN amplified tumour. Thiotepa-related toxicity was mainly digestive with a grade >2 mucositis and diarrhoea in 14 and 16 patients, respectively. Hospitalisation duration was 25 days (19-49). Bu-Mel was administered in 18 patients since 4 patients had a progressive disease after thiotepa. Toxicity was digestive with a grade >2 mucositis and diarrhoea in 13 and 7/18 patients, respectively and hepatic with 6/18 hepatic veno occlusive disease. Toxic related death occurred in 1 patient due to alveolar haemorrhage.
The 3-year EFS survival is 42.6% (24-64).
This intensified HDC strategy in VHR patients seems to be feasible and to improve survival. It will be compared to a combined mIBG-Bu-Mel strategy in the future VHR neuroblastoma European Protocol.

**P1007**

**131I-metaiodobenzylguanidine conditioning regimen in children with neuroblastoma undergoing autologous peripheral stem cell transplantation**

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Radio-labelled 131I-metaiodobenzylguanidine (MIBG) therapy is used in the treatment of high risk neuroblastoma as a part of conditioning regimen in combination with other chemotherapeutics. The maximum-tolerated dose of 131I-MIBG was reported as 12 mCi/kg. However, mucositis as well as myelosuppression was the major toxicity. 131I-MIBG may increase the toxicity of the drugs combinated. Herein, we report and discuss I131 MIBG dose and toxicity on two children that were treated with 131I-MIBG, followed by myeloablative doses of carboplatin, etoposide, and melphalan with stem cell rescue.
First case, a 9-month-old female presented with right abdominal palpable mass. She was diagnosed with stage IV neuroblastoma and treated with chemotherapy including cyclophosphamide, vincristine, etoposide, cisplatin, dacarbazine, ifosfamide, and adriamycine for 6 courses in addition to surgical resection resulting with a 2 cms residual right adrenal mass. She underwent autologous bone marrow transplantation (ABMT), including 100 mCi (10 mCi/kg) 131 I-MIBG treatment on day -21 and melphalan 45 mg/m$^2$/day; on day -8 to -5, etoposide 200 mg/m$^2$/day, carboplatine 300 mg/m$^2$/day; on day -5 to -2 . On day +15, she presented with grade 4 mucositis and massive gastrointestinal bleeding without any evidence of engrafment. After all vigorous efforts, she died on day +18.
Second case was a 8-year-old female with stage-IV neuroblastoma. She was treated with same chemotherapy for 6 courses and there was a 4 cms residual mass after surgical resection. She received radiotherapy to the residual mass and underwent ABMT with same conditioning regimen including 100 mCi (3.1 mci/kg) 131-I-MIBG on day -21. On day +7, she developed grade 1 GVHD involving an erythematous skin rash in the face and trunk region. There was no platelet engraftment on day +25 when she developed acute hypertension and intracranial bleeding. She died on day +31.
Treatment with 131I-MIBG in combination with myeloablative chemotherapy and ABMT was reported as a feasible option. Major toxicities reported as grade 2-3 mucositis, hematuria, and bacteriuria. Mucositis was the leading toxicity reported and reached to grade 4 in half of the patients with mucositis. Although mucositis and myeloablation was not observed with 131I-MIBG as a single agent, combination therapies with drugs at myeloablative doses may increase toxicity. Further toxicity studies warranted in children undergoing ABMT with 131I-MIBG conditioning.

**P1008**

**Toxicity of treosulfan in paediatric high-dose chemotherapy regimens**

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Objective: In the last decade, treosulfan has increasingly been used in pediatric high dose chemotherapy (HDCT) regimens prior to autologous or allogeneic hematopoietic stem cell transplantation (HSCT) mainly for its antitumour activity and its favourable toxicity profile compared to busulfan. In this single-institution analysis, we have studied the observed toxicities following treosulfan-containing high dose chemotherapy protocols with the aim to identify risk factors related to patient or disease characteristics as well as to the drug itself.
Methods: 25 patients, age 1-17 years, mean 8.3 years, with solid tumours (21; neuroblastoma 9, Ewing sarcoma 8, soft tissue sarcoma 4) and haematological malignancies (4; acute leukaemia 3, lymphoma 1), were assessed for toxicities within 100 days after autologous or allogeneic HSCT with treosulfan-containing high dose chemotherapy regimens.
Results: All patients (pts) engrafted. Treosulfan was administered according to body surface area (BSA) in a dose range of 36-42 g/m². The equivalent kg BW (body weight)-based dose revealed a wide span of 875-1980 mg/kg. Within the group receiving more than 1400 mg/kg BW (higher dose group), 8/9 children were ≤4years and ≤ 18 kg of weight. The most frequent diagnoses in this higher dose/kg BW group were solid tumours (7/9). Stomatitis grades 3/4 were diagnosed in 14/25 (56%) pts, but in 7/9 (78%) in the higher dose group. Hyperbilirubinemia grades 3/4 and veno-occlusive disease (2 pts) were exclusively observed in the higher dose group. With one exception, respiratory events grade 2 (4/5 pts) were only detected in the higher dose group. Adverse events requiring intensive care unit care occurred in 3 pts, 2 of these in the higher dose group. In the higher dose group 3/9 (33%) pts have relapsed or are dead, in the lower dose group 13/16 (81%).
Conclusions: In children above a body weight of 18 kg, calculating treosulfan according to BSA is associated with a favourable toxicity profile. In children with a BW of <18 kg a BSA-based dosing schedule is associated with higher toxicities. Body weight adapted dosing schedules need to be established for this group of younger patients. More patients in the higher dose group were in remission at the time of evaluation; however, in this cohort of heterogeneous diagnoses and treatment protocols no further conclusion is possible.

**P1009**

**Neurotoxicity of high-dose thiotepa in children with solid tumours**

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Objective: To assess acute neurotoxicity occuring in children with solid tumors treated with high-dose thiotepa (600, 720 and 900 mg/m$^2$) followed by autologous stem cell transplantation (ASCT).
Patients and Methods: Patients with solid tumors treated with high-dose thiotepa with ASCT at Institute Gustave Roussy between May 1987 and March 2011 were retrospectively identified through the pediatric transplantation database. Clinico-biological data were collected and neurological adverse events (NAE) were identified from medical and nursing records. Toxicity was graded according to the NCI CTCAE v4.03 classification.
Results: 251 patients received 307 courses (56 patients received twice) of high-dose thiotepa (600 mg/m$^2$: 82 courses; 720 mg/m$^2$: 76 courses; 900 mg/m$^2$: 149 courses) with ASCT for a solid tumor. The median age was 8.33 years (range, 1-31.2 years). 67 NAE (22%) were described in the nursing records, and 37 (12%) in medical records. In 22 courses, NAE were considered possibly related to thiotepa. In these 22 NAE, neurological symptoms appeared at a median time of 2 days (range, 0-4 days) after the introduction of thiotepa. The neurological symptoms were tremor in 4 patients, seizure in 6 patients, pyramidal tract syndrome in 2 patients, cerebellar syndrome in 3 patients, opsoclonus-myoclonus syndrome grade 2 in 1 patient, headache, dizziness and confusion in 4 patients, blurred vision, diplopia, nystagmus and eye pain in 4 patients, and coma in 1 case. These events disappeared without sequelea in a median time of 3 days (range, 1–8 days). After these NAE, thiotepa was reintroduced in 7 cases. One patient had tremor grade 2 and syncope grade 3, one child had headache grade 3. Three patients who presented seizure during the first course of thiotepa, received clonazepam during the second course. Thus, reintroduction of thiotepa was carried out without NAE for 5 of 7 patients.
Conclusion: In our study, the incidence of NAE related to thiotepa was 7.2%. The evolution was favorable without sequelea in all cases. Thiotepa could be reintroduced after NAE. To continue our research, we will perform a correlation analysis to find potential risk factors to develop NAE related to thiotepa.

**P1010**

**Quantification of sulfolane, a metabolite of busulfan in plasma by gas chromatography and tandem mass spectrometry: a new method for evaluating the role of sulfolane and metabolic fate of busulfan**

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Busulfan (Bu) is commonly used as a component of conditioning regimen in pediatric patients before hematopoietic stem cell transplantation (HSCT). Bu undergoes metabolism by conjugation with glutathione conjugating enzymes yielding intermediary unstable metabolites sulfonium ion and tetrahydrothiophene (THT). Tetrahydrothiophene undergoes oxidation into sulfolane (Su) which is relatively stable than THT. The role played by these metabolites on the events seen during busulfan treatment is not completely known. Lack of established analytical methods for estimation of busulfan metabolites limits our understanding of their role. Measurement of unstable metabolites may not be possible for routine investigations, because of the special precautions and procedures. Hence, we attempted to develop a new method for the measurement of Su by gas chromatography/tandem mass spectrometric assay (GC-MS/MS) along with Bu. Bu and Su were simultaneously extracted from 100 micro liters of plasma by ethyl acetate. Two separated GC runs were developed for Bu and Su. MS detection of the analytes was performed in the selected reaction monitoring mode on a triple quadrupole instrument after electronic impact ionization. The method was validated for the concentration range of 50–2000 ng/mL for Bu and 40–400 ng/mL for Su. In a preliminary study, clinical samples taken from two children after doses 1, 2, 3, and 9 showed to have detectable levels of Su in plasma starting from four hours after the first dose. We observed increased levels of Su after the 9th dose of Bu (greater than 200 ng/mL) in the plasma of a children receiving Bu prior to HSCT. Later, sulfolane was analyzed in EBMT cohort plasma samples collected 4 hours after 1st dose (n=46), before 7th dose (n=57) and after 9th dose infusion (n=54). We observed mean sulfolane values of 24.6, 181 and 249.9 ng/mL, at 4 hours after 1st dose, before 7th dose and after 9th dose infusion, respectively. The mean busulfan levels measured at these three time points were 266 (n=68), 393 (n=63), and 1271 ng/mL (n=57). Clinical studies may adopt this novel method for investigating busulfan metabolic fate and its mechanisms of toxicity. Understanding the kinetics of sulfolane in subjects undergoing busulfan infusion and its role in the adverse effects seen with busulfan is possible with this method.

**P1011**

**Haematologic recovery in tandem high-dose chemotherapy and autologous stem cell transplantation in children with high-risk solid tumours: platelet and RBC recovery are significantly delayed in the second transplantation**

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Background: Recently, reports employing tandem high-dose chemotherapy and autologous stem cell transplantation (HDCT/autoSCT) for the treatment of high-risk pediatric solid tumor are increasing. Stem cell dose in tandem HDCT/autoSCT is generally lower than that in single HDCT/autoSCT. However, data about hematologic recovery during tandem HDCT/autoSCT, particularly in relation to CD34+ cells dose, are very limited to date.
Patients and Methods: We retrospectively analyzed the hematologic recovery in 236 children with high-risk solid tumors who underwent tandem HDCT/autoSCT between April 1998 and June 2011.
Results: The median numbers of CD34+ cells transplanted in the first and second HDCT/autoSCT were 4.24x10$^6$/kg and 3.99x10$^6$/kg (P=0.653). There was no difference in neutrophil recovery between the first and second HDCT/autoSCT (median 10 days *vs.* 10 days to reach absolute neutrophil count of 500/uL, P=0.134). However, platelet and RBC recovery were significantly delayed in the second HDCT/autoSCT. The median time to reach a platelet count of 20,000/uL without transfusion for the previous 7days in the first and second HDCT/autoSCT were 22 (range 8-79) days and 28 (range 13-596) days, respectively (P<0.001). The median time for RBC recovery (first day of hemoglobin ≥8.0 g/dL without transfusion for previous 28 days) in the first and second HDCT/autoSCT were 40 (range 30-132) days and 44 (range 31-452) days, respectively (P=0.001). Similarly, the number of platelet and RBC transfusion were higher in the second HDCT/autoSCT than in the first HDCT/autoSCT (median 7 *vs.* 9, P<0.001; median 3 *vs.* 4, P=0.001). Differences in hematologic recovery and transfusion requirement were more significant when the analysis was confined to patients who were transplanted with CD34+ cells less than 5x10$^6$/kg, particularly less than 2x10$^6$/kg. Accordingly, ferritin levels at 1 year after tandem HDCT/autoSCT were higher in patients who were transplanted with CD34+ cells less than 2x10$^6$/kg (median 1149.2 *vs* 739.65 mg/mL, P<0.004).
Conclusion: Our results strongly support the notion that further modalities of collecting more CD34+ cells must be researched

in order to accelerate hematologic recovery after tandem HDCT/autoSCT, reduce transfusion amount, lower ferritin level, and possibly decrease organ dysfunction related to iron overload in patients with high-risk solid tumors.

**P1012**

**Allogeneic haematopoietic stem cell transplantation for primary immunodeficiency diseases in Korea: a single-centre experience**

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Objectives: Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) has been considered a curative treatment modality for primary immunodeficiency diseases (PIDs), data on allo-HSCT for PIDs are scarce in Korea. This study was aimed to evaluate the feasibility of allo-HSCT for various PIDs.
Methods: We have performed 14 transplants for 12 patients with PIDs. For X-linked severe combined immune deficiency (X-SCID), small volume of donor marrow was infused without conditioning and graft-versus-host disease (GVHD) prophylaxis. For non-SCID, busulfan-based conditioning regimen was applied and GVHD prophylaxis was variable according to the source of stem cells.
Results: The underlying diseases were X-SCID (n=3), Wiskott-Aldrich syndrome (WAS; n=3), hyper-IgM syndrome (HIGM; n=2), chronic granulomatous disease (CGD; n=2), and familial hemophagocytic lymphohistiocytosis (FHL; n=2). An HLA-identical sibling donor was available in 6 patients and the other half received grafts from alternative donors. Their median age at transplant was 3.6 y (range, 0.3-13.2). Nine patients achieved sustained engraftment, one of whom died of severe chronic GVHD. Of 3 patients who had poor graft function, one died of cytomegalovirus pneumonia and one of the other two who received second transplantation was successfully rescued. All 3 deaths exclusively occurred among those who received alternative donor graft. Ultimately, 9 out of 12 patients are alive disease-free with a median follow-up of 56 mo (range, 2-167). All X-SCID patients achieved normal lymphocyte counts and immunoglobulin levels as well as normal lymphocyte proliferative response to mitogen. Two CGD patients showed normal dihydrorhodamine test at 2 mo post-transplant.
Conclusion: Allo-HSCT was feasible as a curative modality for both SCID and non-SCID. Given the high transplant-related mortality following alternative donor transplants, whether transplant or not should be determined carefully after weighing the pros and cons especially if matched familial donor is not available.

**P1013**

**Haematopoietic stem cell transplantation in primary immunodeficiency diseases: the Iranian experience**

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Objectives: Primary immunodeficiency diseases (PID) are rare but associated with high mortality rate due to infectious problems. As the vast majority of PID patients are affected by infection at the time of transplantation and the use of myeloablative conditioning regimens may lead to higher mortality rate, we conducted a prospective study of reduced-intensity conditioning (RIC) as preparation for patients.
Methods: Twenty-eight patients (8 female, 20 male) who had undergone allo-HSCT between 2006 and 2010 were enrolled in the study. Eleven patients received stem cell from healthy full human leukocyte antigen (HLA) matched siblings, 7 from HLA matched other related donors (4 patients from parents, 2 from grandfather and one from uncle), three from their haploidentical parents and 7 from unrelated donors. Stem cells were collected from the bone marrow (n=7), peripheral blood (n=14) and cord blood (n=7). Median age at HSCT was 19 months (4 months-14 years). The various types of diseases observed in patients were Leukocyte Adhesion Deficiency type one (n=12), Wiskott Aldrich Syndrom (n=6), Griscelli syndrome (n=3), Severe Combined Immunodeficiency (n=2), Chediak-higashi, Familial Erythrophagocytic Lymphohistiocytosis and primary CD4 deficiency (n=1). All patients were conditioned with a reduced intensity conditioning regimen containing fludarabine, melfalan and antithymocyte globulin. For graft-versus-host disease (GvHD) prophylaxis, cyclosporine plus prednisolone at the standard doses were used.
Results: All patients but one engrafted successfully. 24 (85.7%) patients with sustained engraftment were alive with a median follow-up of 9 months. No evidence of recurrent infection was seen among these patients. The causes of death among 5 patients were acute GvHD (n=3), non-engraftment infectious complications (n=1) and aspiration pneumonia (n=1).
Conclusions: The PID patients can take advantage from HSCT, but early diagnosis is important for rescue patients from lethal disease complications. The use of reduced-intensity conditioning regimen in the PID patients who suffer from co-morbidity at the time of transplantation, may reduce the treatment-related mortality (TRM).

**P1014**

**Haploidentical stem cell transplantation with CD3/CD19 depleted grafts in paediatric patients with aplastic anaemias and haemoglobinopathies**

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We investigated a cohort of 13 pediatric patients with non-malignant diseases transplanted with T- and B-cell depleted peripheral stem cell grafts from haploidentical donors between 2004 and 2011. 5 patients had myelodyslastic syndrome with refractory cytopenia (MDS-RC), 5 had severe aplastic anemias (SAA) and 3 patients had hemoglobinopathies. 3 out of 13 patients received a 2nd SCT after rejecting the graft from matched related or unrelated donors. Median age was 8.3 years. Standard conditioning regimen consisted of Fludarabin (4x40 mg/m$^2$), Thiotepa (1x10 mg/kg), Melphalan (2x70 mg/m$^2$) and OKT3. Since 2009, patients received upfront additional total lymphoid irradiation (TLI, n=3) to prevent graft rejection. In the current protocol, OKT3 will be substituted by ATG. Graft manipulation was carried out by direct depletion using antiCD3/19 coated magnetic microbeads. A median number of 15.6x10$^6$ CD34+ Progenitorcells and 53x10$^3$ T-cells/kg body weight (BW) were transfused. Pharmacological Graft *vs*.Host Disease (GvHD) prophylaxis was carried out with Mycofenolate until day 60, if residual T-cells in the graft exceeded 25000/kg BW. Primary engraftment occurred in all patients. 5/10 patients with non irradiation regimen rejected the haplo graft and needed reconditioning with Fludarabine (3x40 mg/m$^2$), Thiotepa (1x5 mg/kg), ATG, OKT3, 7 Gy TLI and a second stem cell donation from a different parental donor. Thus, final engraftment was achieved in 13/13 patients. None of the patients rejected the second graft. None of the patients receiving upfront preventive TLI rejected the first graft. Median time to reach 500/µl neutrophiles was 9 days (9-14). Independence from platelet substitution was reached after 11 days (8-16). 7/13 patients (54%) had no signs of GvHD or GvHD grade I, 5 patients (38%) had GvHD grade II, 1 patient developed grade III (8%). TRM at day+100 and after 1 year was 0% and 15%, respectively. Event free survival (EFS) at 3 years was 85%.

Conclusions: Haploidentical SCT with T-cell depleted grafts is a therapeutic option for non-malignant diseases if no matched donor is available. TRM was low, even if retransplantation was necessary. Fast recoveries of neutrophiles and platelets were achieved. Rejection could be avoided by adding TLI to the initial conditionig regimen. If a non-irradiation regimen is used, possible graft failures can be rescued by reconditioning with TLI and a second stem cell donation from a different parental donor.

**P1015**

**Mixed chimerism has no impact on survival in children with severe aplastic anaemia post stem cell transplantation**

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Aim: In the retrospective study we evaluated the clinical impact of chimerism after allogeneic stem cell transplantation (alloHSCT) in children with severe aplastic anemia (SAA).
Patient and Methods: Between 2000 and 2010 twenty five children suffering from SAA was referred to our center for transplantation. There were 14 boys and median age of the group was 9 years (range 8 months to 15 years). Transplant from match related donor bone marrow was performed in 14 children and alloHSCT was primary treatment of these patients. Eight children was transplanted from match unrelated donors (MUD) and the procedure was introduced as a salvage therapy after failure of immunosuppressive therapy (IST). One patient was transplanted from haploidentical donor and one patient received sibling graft after IST. Post transplant chimerism was evaluated at following time periods: 1, 3, 6, 12 months post alloHSCT and at least once a year therefore. Multiplex short tandem repeated PCR (STR-PCR) was used as a method of chimerism evaluation.
Results: 21/24 patients engrafted and 3/20 did not show engraftment (2 unrelated donor and one sibling donor after IST) and one boy was rescued by the second graft from the same unrelated donor. Three patients died from transplant related complication: two girls died from multi-organ failure caused by infections (one before engraftment and one after re-transplantation) one girl died from lymphoproliferative disease which occurred after re-transplantation and IST failure. 22/25 children are alive, in good clinical and hematological condition, median 5 years after transplantation (range 1-10 years).
One patient (4%) experienced autologous recovery after MSD alloHSCT. In 9/21 (43%) survivals we observed full donor chimerism during observation time. Eleven patients (53%) developed mixed chimerism, ranging from 6,4 to 39 percent recipient cells in peripheral blood. Mixed chimerism increased in eight patients and in three children was stable. 3/24 patients (2 MUD and 1 MUD) after primary engraftment rejected their graft 2,3 and 22 months after alloHSCT and 2 of them were successfully re-transplanted from the same donor. Remaining eight patients have normal peripheral blood count despite having mixed chimerism. All surviving patients are off immunosuppression, without symptoms of GvHD.
Conclusion: In our material mixed chimerism does not impair the outcome of children with Severe Aplastic Anemia after alloHSCT.

**P1016**

**A fludarabine-based conditioning for alternative donor haematopoietic stem cell transplantation in inherited bone marrow failure syndrome**

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Objective: Inherited bone marrow failure syndrome (IBMFS) is a group of heterogeneous disorders characterized by cytopenias usually in association with one or more physical malformations. We report the outcome of 11 IBMFS patients (pts), who were given alternative donor hematopoietic stem cell transplantation (HSCT) using low-dose irradiation and fludarabine-based conditioning.
Methods: Between 1/2002 and 7/2011, 11 IBMFS pts received HSCT. They are all excluded from Fanconi anemia and Diamond-Blackfan syndrome by clinical features, chromosome instability and/or genetic subtypes. Three children had dyskeratosis congenita and other eight were unclassifiable IBMFS by Canadian Inherited Marrow Failure Registry criteria. There were 5 males and 6 females, aged 10 months to 11.8 years old at HSCT. Hematological diagnosis included transfusion dependent severe aplastic anemia (n=10) and RCMD (n=1). Donors were HLA- matched unrelated (n=4), mismatched unrelated (n=5), haploidentical mother (n=1) and matched unrelated cord blood (n=1). Conditioning regimen was TAI (3Gy) + fludarabine + CY ± ATG, which is used as a conditioning for acquired aplastic anemia or RA in our hospital. GVHD prophylaxis included tacrolimus + MTX ± MMF for all pts.
Results: All pts engrafted. None of the pts showed grade III/VI regimen-related organ toxicity using the Bearman criteria. Acute GVHD, I, II, and chronic GVHD were observed in 2, 3, 3 pts, respectively. All pts remain alive and well at a median of 51 months following HSCT (range 4-118mo). Conclusion: Our study indicates that significant advances have been made in the use of alternative HSCT to treat IBMFS using low-dose irradiation and fludarabine-based conditioning regimen. Furthermore, the low dose ATG administered prior to transplant might exert a preventive effect on GVHD in conjunction with tacrolimus and short-term MTX.

**P1017**

**Improved outcome of stem cell transplantation in children with congenital bone marrow failure syndromes – the use of fludarabine with low-dose cyclophosphamide**

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Fludarabine based protocols are increasingly being used as conditioning regimens in patients with aplastic anemia. Between 1999 and 2011, 22 children (15 male and 7 female) with CBMFS [20 with Fanconi anemia (FA) and 2 with Dyskeratosis congenital (DKC)] underwent HLA identical stem cell transplantation (HSCT) using a fludarabine based protocol. Sixteen children with FA had typical morphological abnormalities and were positive by stress cytogenetics while 4 children had morphological abnormalities but were stress negative. Both patients with DKC were diagnosed clinically. Nineteen children had an aplastic marrow while 3 patients with FA had myelodysplastic syndromes on bone marrow analysis. The conditioning protocol consisted of Fludarabine (180 mg/m$^2$ over 6 days), Cyclophosphamide (20 mg/kg over 2 days) + Anti-thymocyte globulin (40 mg/kg over 4 days). In patients with FA and MDS, cyclophosphamide was replaced by busulfan (6 mg/kg).

Cyclosporine with mini methotrexate was used for GVHD prophylaxis. Graft source included peripheral blood stem cells (15) or G-CSF stimulated bone marrow (7).
All patients engrafted (100%) with a median time to neutrophil engraftment of 14 days (range: 9–29) and a median time to platelet engraftment of 12 days (range: 9–31). The incidence of hemorrhagic cystitis and veno-occlusive disease was <5%. The incidence of grade II-IV acute graft versus host disease (GVHD) was 28.5% with chronic GVHD seen in 40% (predominantly limited). One patient with FA and 2 with DKC had secondary graft failure while one patient relapsed as AML; 3 of these patients subsequently underwent a second transplant using the same donor. Five children have expired leading to a 2 year survival of 77.2%.
Fludarabine based protocols are associated with improved survival in children with congenital bone marrow failure syndromes.

**P1018**

**Treosulfan-based conditioning in 6 pediatric and adolescent patients with non-malignant diseases and haematological malignancies**

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Introduction: Treosulfan demonstrates cytotoxic/antiproliferative activity against a wide range of malignancies, as well as myeloablative and potent immunosuppressive effects, and has a favorable toxicity profile. Treosulfan-based preparative regimens for allogeneic hematopoietic stem cell transplantation (HSCT) have been used in children with hematological malignancies and non-malignant disorders. We report our experience on 6 patients receiving treosulfan-based conditioning between 2009 and 2011.
Patients and Methods: Diagnoses of the 6 patients (m:f=4:2; median age: 2.1 years; range: 0.5-23.2 ) were: Hurler's disease (n=2), X-linked-lymphoproliferative syndrome (XLP), Griscelli syndrome type 2, high-risk acute lymphoblastic leukemia (CR1), relapsed juvenile myelo-monocytic leukemia. Donors were matched unrelated (n=3), haploidentical parents (n=2) and one matched sibling. Grafts were peripheral blood stem cells (PBSC; CD3/19 depleted ± CD34 selected) in 4 patients, bone marrow (BM) in 1, and BM plus PBSC in 1 patient, containing in median 19.8 x$10^6$ CD34+/kg. Conditioning consisted of Treosulfan (14 g/$m^2$: n=5; infant:12 g/$m^2$), Fludarabine (150-150 mg/$m^2$), Thiotepa (7.5-10 mg/kg) and ATG Fresenius (n=5: 30-60 mg/kg) or Thymoglobulin (n=1: 3 mg/kg x2).
Results: Leukocyte engraftment (WBC>1.0 G/l) was achieved by all patients on median day +13.5 (+9 to +19), platelet engraftment by 5 patients on day +10 (+8 to +13). Five patients established a stable donor chimerism of ≥95% from median day +10. The infant with Griscelli syndrome experienced reactivation of hemophagocytosis around day +13 leading to graft rejection and is being prepared for second HSCT. Prophylaxis of graft-versus-host disease (GVH) consisted of Mycophenolate-mofetil (n=4) or Cyclosporine A (n=2); no signs of acute or chronic GVH were noted so far. Acute extramedullary toxicity was mainly moderate mucositits (n=3), diarrhea (n=1) and mild skin toxicity (n=2). All patients are alive for a median of 13.5 (1-31) months. During immune reconstitution (IR) the patient with XLP developed IR-inflammatory syndrome leading to permanent CNS compromise.
Conclusions: Treosulfan-based conditioning lead to stable hematopoietic engraftment with early complete donor chimerism in 5/6 patients including 3 patients with nonmalignant diseases with mild acute toxicity but was not able to prevent early reactivation of hemophagocytosis leading to graft rejection in the patient with Griscelli syndrome.

**P1019**

**Fludarabine as second-line treatment in paediatric steroid refractory allo-immune lung syndromes**

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Objectives: Allo-immune lung syndromes (alloLS) as bronchiolitis obliterans syndrome (BOS), bronchiolitis obliterans organiz-

**[P1019]**

**Table 1 Patient characteristics at start alloLS for which Methylprednisolone pulse**

| Patient | Indication | Age (yrs) | Gender | TX (type) | Time after TX (months) | Diagnosis | Diagnostic tests | Virology (CT) |
|---|---|---|---|---|---|---|---|---|
| 1 | Relapsed Infant ALL | 2 | F | HSCT Cord 05/06 | 2 | AIP>BOS | CT: Mosaic attenuation without airtrapping, ground glass opacities, tree in bud sign, small centrilobular nodules, septal lines | BAL and NPA: Rhino (17) |
| 2 | JMML | 1 | F | HSCT Cord 05/06 | 2 | IPS | CT: Bilateral consolidations with ground glass opacities | BAL and NPA: Adeno (22) |
| 3 | Hurler syndrome | 1 | M | HSCT Cord 04/06 | 2 | AIP>BOS | CT: Ground glass opacities, small noduli (halo), some aitrapping (also seen on the pre HSCT CTscan) | BAL and NPA: Neg |
| 4 | Cutaneous lymophoma associated with CID | 15 | M | HSCT MUD 09/10 | 8 | IPS>BOOP | CT: Diffuse infiltrates mainly located peripherally PFT: FVC 46.2% (pre HSCT103.8%), FEV1 47.4% (pre HSCT102.1%) | BAL: Rhino (18) & Galactomannan |
| 5 | Surfactant C deficiency | 6 | M | LTX | 4.5 | BO(S) (biopsy) | CT: not performed PFT: FEV1 17% (pre LTX 30%) | NPA: Parainfluenza (21/42)* & RSV (28) |

AlloLS allo immune lung syndromes, ALL acute lymphatic leukemia, JMML juvenile myelomonocytic leukemia, CID combined immunodeficiency, F female, M male, HSCT hematopoietic stem cell transplantation, LTX lung transplantation, AIP acute interstitial pneumonitis, IPS idiopathic pneumonia syndrome, BOS bronchiolitis obliterans syndrome, BOOP bronchiolitis obliterans organizing pneumonia, HRCT high resolution CT scan, PFT pulmonary function test, NPA nasopharyngeal aspirate, BAL bronchoalveolar lavage, CT cycle tresholds, Neg negative, RSV respiratory syncitial virus
* Parainfluenza was detected at start of the pulmonary symptoms but during therapy with steroids the viral load resolved (CT 42)

[P1019]

**Table 2 Disease and patient characteristics after treatment with fludarabine**

| Patient | Indication | Time after TX of first Fludarabine (months) | Courses of Fludarabine | Response alloLS | Virology (CT) | Outcome |
|---|---|---|---|---|---|---|
| 1 | Relapsed Infant ALL | 6 | 4 | Complete: no pneumonia symptoms or signs | BAL and NPA: Rhino (19) | Died of disease (1.5 yrs after last Fludarabine) |
| 2 | JMML | 4 | 6 | Complete: no pneumonia symptoms or signs | NPA: Neg | Alive |
| 3 | Hurler syndrome | 3 | 2 | Partial: overnight mechanic ventilation for hypercapnia. Diagnosed with BO | BAL: Neg | Alive |
| 4 | Cutaneous lymophoma associated with CID | 10.5 | 4 | Complete: FVC 84.3% & FEV1 78.6%, though HRCT with ground glass opacities | BAL: Rhino (27/22) | Died of disease (lymphoma and CID, 5 months after last Fludarabine) |
| 5 | Surfactant C deficiency | 5.5 | 3 | Partial: decrease in supplementary oxgygen, less dyspnea and increase in FEV1 (17 -> 21%) | NPA: Rhino (19/42)** | Died of progression after initial response (2 months after last Fludarabine) |

** Rhinovirus load was detected just before the second Fludarabine but could not be detected anymore after the second and third course

ing pneumonia (BOOP) and idiopathic pneumonia syndrome (IPS) are life-threatening complications after either hematopoietic stem cell (HSCT) or lung transplantation (LTX). Respiratory viral infections are suggested to trigger these alloLS.
Methods: In patients with steroid refractory alloLS (no improvement or progressive alloLS despite methylprednisolone) the methylpredisolone-pulses were replaced by fludarabine as immunosuppressive treatment. Corticosteroids were tapered off while fludarabine was given in a dose of 30 mg/m$^2$ every three weeks, for a maximum of 6 courses. Every time response to fludarabine gifts was noted, another course was scheduled. Response was defined as improvement/normalization of PFTs compared to baseline pre-HSCT and/or resolved respiratory symptoms, with either reduction or no extra oxygen requirement.
Results: 5 patients were included: all (4 HSCT and 1 LTX) showed good allogenic donor cell engraftment and/or function of the graft. Patients developed respiratory signs and symptoms of alloLS 2 months (median time) after transplantation (range 2-8 months) for which radiologic and pulmonary function tests were performed (Table 1). These 5 patients with eventually steroid refractory alloLS were treated with fludarabine. Fludarabine was started at a median of 5.5 months after transplantation (range 3-10.5 months). All showed either partial or complete fludarabine response after a median of 4 courses (range 2-6 courses) (Table 2). Despite a high load of respiratory viral infections none of these patients showed progression of viral disease.
Conclusion: Fludarabine is a feasible and an effective second line treatment of steroid refractory alloLS, without an increase in viral load and possible associated viral disease.

**P1020**

**Reduced-intensity conditioning followed by matched unrelated donor bone marrow transplant in a 2.5 year-old male with chronic granulomatous disease with one functioning lung**

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Chronic Granulomatous disease (CGD) is an inherited disorder of the NADPH oxidase characterized by severe bacterial and fungal infections and excessive inflammation affecting 1 in 200,000 persons. Even with the best antimicrobial prophylactic treatment, patients experience frequent hospitalizations and only 50% of patients are alive at 30 years.
Bone Marrow Transplant (BMT) provides curative therapy for patients with CGD, although controversy exists over the requirement for BMT in all patients, and the optimal timing for any BMT procedure.
This patient was diagnosed at age 16 months with X linked CGD (CYBB gene mutation)after poor response to antimicrobial therapy for bilobar pneumonia. After no clinical response following 3 months of antimicrobial therapy he was referred to the surgical team for decortication by VATS procedure at age 19 months. Pulmonary complications included ECMO therapy, mechanical ventilation and several surgical procedures. He was treated on an outpatient basis with a 9 month prolonged combination of three antimicrobial agents and steroid pulses. He was observed off antimicrobial therapy for 6 months followed by serial ESR and CRP biweekly.
He was then brought to Reduce Intensity Conditioning (RIC) Matched Unrelated Donor (MUD) BMT utilizing distal Alemtuzumab days -23 to -21 at 10, 15 and 20 mg followed by 150 mg/m$^2$ of Fludarabine over 5 days (-8 to -4) and 140 mg/m$^2$ of Melphalan on Day -3.
He received 12/12 MUD Bone Marrow at TNC of $4.35\times10^8$/m$^2$. GVHD prophylaxis used Methotrexate 7.5 mg/m$^2$ Days +1,3 & 6 along with Tacrolimus and Methylprednisolone. Methylprednisolone was started at Day +7 and initially planned to taper off by day +56 but prolonged to Day + 90 due to skin GVHD.
He tolerated the transplant well with the one functioning lung. He engrafted neutrophils at Day +13 and at 6 month follow up maintains a 100% donor chimerism with a normal dihydrorhodamine-1, 2, 3 test.
Reduce Intensity Conditioning with subsequent Bone Marrow Transplant is a promising treatment modality for fragile CGD patients and should be considered and offered even in the Matched Unrelated Donor setting.

**P1021**

**The effect of glutamine supplementation in children who underwent haematopoietic stem cell transplantation**

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Glutamine (Gln) is a conditionally essential amino acid during high dose chemo-radiotherapy especially for enterocytes. It is

unclear whether supplemental glutamine is of benefit in hematopoietic Stem Cell Transplantation (HSCT). Reviews and guidelines in the area have given conflicting advice. We therefore conducted a retrospective study to determine the effect of oral Gln supplementation in children undergoing HSCT. A retrospective study of 76 (42 male, 34 female) HSCT recipients with a mean age of 8 years (1,5-18), who received myeloablative regimen at our center between July 2001 and October 2011 was undertaken. Of these patients 19 didn't receive Gln (control group), 25 received 1 g/m²/day (group 1), 32 received 2 g/m²/day (group 2) oral Gln. Gln was given beginning on the day of myeloablative regimen until discharge as swish and swallow administiration every twelve hours. Patients were examined daily and OM was graded, based on WHO scale. Analyses examined the difference of clinical outcomes between groups including beginning, incidence and duration of the OM, incidence and duration of fever and diarrhea, number of documented infection, neutrophil engraftment, aGVHD, sinusoidal obstruction syndrome (SOS), inpatient days and mortality rates. Patients in the supplemented group (group 2 and 1) had statistically significant shorter fever duration than patients in the control group (4,0 ± 2,4; 4,2 ± 2,7 and 6,0 ± 2,7 days respectively; p = 0.001). Patients in group 2 had statistically significant lower SOS incidence than patients in the group 1 and control group (0/32; 2/25; 5/19 respectively; p=0.031). There were no statistically significant difference between the groups in OM and also in the other selected outcomes above. Gln doesn't appear to be beneficial in reducing mucositis and most of the other complications of HSCT. In our study, the duration of fever was found to be significantly decreased in the supplemented group. However, there was no significant difference in the incidence of documented infection between groups. Several reports like ours suggest beneficial effects of gln supplementation in HSCT in protection from SOS. But there are also studies reporting increased relapse risk with glutamine. Careful consideration should be given to include oral Gln supplementation as a routine part of supportive care of children underwent HSCT.

**P1022**

**Role of serotherapy for SCT in familial haemophagocytic lymphohistiocytosis**

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Background: Familial Hemophagocytic Lymphohistiocytosis (FHL) is a congenital immune deficiency, resulting from biallelic mutations in the PRF1 (FHL2), Munc13-4 (FHL3), Syntaxin11 (FHL4), STXBP2 (FHL5) genes which cause variable defects of the cellular cytotoxicity machinery. Chemo-immunotherapy allows temporary disease control, but HSCT holds as the only procedure with potential for cure. Myeloablative conditioning regimens have been largely used in the past, providing stable graft in the majority of patients but at the price of relevant toxicity. To minimize the TRM, reduced intensity conditioning regimens were introduced, but this was associated with a higher proportion of patients resulting in mixed chimerism. Thus, the selection of the conditioning regimen with or without immunotherapy may turn to be crucial for the outcome of SCT and final cure.
Results: The main features of the four female children, consecutively treated in our center, are summarized in the table. GVHD prophylaxis was based on cyclosporine + methotrexate; methotrexate was omitted in case 1, with UCB donor. All children are alive after a follow-up time comprised between 3 months and 3 years.
None of the children developed VOD nor FHL reactivation. Yet, the only patient who did not receive serotherapy (either ATG or Alemtuzumab) developed mixed chimerism with a residual proportion of donor cells of 10%, despite DLIs.
Conclusion: In our experience serotherapy contributed to successful engraftment and disease control in FHL. Optimal dose and time of administration should further addressed.

**P1023**

**Factors influencing engraftment in children with benign diseases: single-centre experience from Turkey**

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Hematopoetic stem cell transplantation (HSCT) is an effective treatment modality for a range of life-threatening diseae in children. The long-term survival rates in children and adolescents have been increased over last years. However, graft failure is still an important problem affecting morbidity and mortality. In the present study, we therefore analysed factors influencing engraftment in paediatric HSCT for benign disease.
Patients and Methods: We retrospectively evaluated 240 pediatric patients who underwent HSCT at Akdeniz University Pediatric Bone Marrow Transplantation Unit for benign diseases from 1998 to June 2011. Median age was 7 years (range: 0.3-29). Male/Female ratio was 128/112 (53/47%). The diagnosis of the patients were seen in (Table 1). Donors were siblings in 64.2%,

**[P1022]** MAIN FEATURES OF FOUR CHILDREN WITH FHL TREATED WITH SCT

| Case # | Diagnosis | Age at diagnosis | Time to SCT | Conditioning regimen | Donor, source | Chimerism % donor |
|---|---|---|---|---|---|---|
| 1 | FHL3 | 15 mos | 2 mos | Treosulfan, fludarabine, ATG | Unrelated cord blood | 70% |
| 2 | FHL2 | 19 mos | 3 mos | Treosulfan, fludarabine, | Matched familial donor, BM | 10% |
| 3 | FHL2 | 7 mos | 4 mos | Melfalan, fludarabine, distal alemtuzumab | Matched unrelated donor, PBSC | 100% |
| 4 | FHL3 | 10 years | 4 mos | Melfalan, fludarabine, distal alemtuzumab | Matched unrelated donor, BM | 100% |

[P1023] **Table 1. The diagnosis of patients**

| Diagnosis | The number of patients (%) |
|---|---|
| Thalassemia major | 134 (55.8) |
| Fanconi anemia | 27 (11.2) |
| Aplastic anemia | 21 (8.7) |
| SCID | 11(4.6) |
| ALD | 7 (2.9) |
| Hurler syndrome | 5 (2.1) |
| Osteopetrosis | 5 (2.1) |
| WAS | 4 (1.7) |
| HLH | 4 (1.7) |
| CAMT | 3 (1.3) |
| Griscelli syndrome | 3 (1.3) |
| Kostman syndrome | 3(1.3) |
| DBA | 2 (0.8) |
| CHS | 2 (0.8) |
| Myelofibrosis | 2 (0.8) |
| Others* | 7 (2.9) |

* One each patient with congenital neutropenia, LAD, KGD, mannosidosis, fukosidosis, Sβ thalassemia, evans syndrome.

matched family in 24.6% and 11.2% were MUD. Stem cell source was PBSC in 60%, BM in 23.8% and CB 10.4%. Neutrophil and platelet engraftment was developed median 14 days (range 8-109 days) 19 days (range 7-229 days), respectively. Graft failure was developed 7.2% of patients. We analysed risk factors influencing engraftment. In univariate analysis; HLA-match was associated with increased risk of engraftment (p=0.005). While 10/10 HLA-matched transplants compared with HLA-1 mismathed, HLA-matched transplants showed better engraftment rate (p=0.005). In cord blood group, engraftment rate was lower (BM & CB p=0.003; PBSC & CB p=0.01). In cord blood group, graft failure was seen 25%. While in BM group, TNC and CD34+ cell dose were associated high engraftment rate (p=0.001); in PBSC group CD34+ cell dose was found more significant (p=0.001). In cord blood group; CD34+ cell dose was more important for engraftment (=0.002). However, there is no difference in multivariate analysis. The gender, patient's age, donor's age, conditioning regimen, mismatch locus, ABO mismatching and patient-donor CMV sero status have no effect on engraftment. We didn't find any difference between MSD and MUD transplant results about engraftment.
Conclusion: Graft failure is still contributing factor to morbidity and mortality of HSCT. This study suggested that HLA-matching, stem cell source and cell dose are influencing factors on engraftment in patients with benign disease. Cord blood seems to be an important factor to development of graft failure. Further studies are needed to find risk factors influencing engraftment.

**P1024**

**Neurological complications following haematopoetic stem cell transplantation in children with benign diseases: single-center experience from Turkey**

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Haematopoietic stem cell transplantation (HSCT) is an effective treatment modality for a range of life-threatening diseae in children. The long-term survival rates in children and adolescents have been increased over last years. Neurological complications are considered an important cause of treatment-related morbidity and mortality after HSCT. In the present study, we therefore analysed incidence and etiological factors of neurological complications following paediatric HSCT.
Patients and Methods: We retrospectively evaluated 240 pediatric patients who underwent allogeneic HSCT at Akdeniz University Pediatric Bone Marrow Transplantation Unit for benign diseases from 1998 to June 2011 for the development of post transplant neurological complications. After HSCT, neurological complications developed at 27 (11.2%) patients. The mean of age was 8.8±5.8 years. Male/female ratio was 14/13. The diagnosis of patients were shown Table 1. Donors were matched sibling donor (MSD) in 6 (51.9%), matched family donor (MFD) in 7 (25.9%), unrelated donor in 6 (22.2%). Peripheral blood stem cell was used in 16 (59.3%) tranplantation. These neurological symptoms were consisted of convulsion (n=19), headache and vomiting (n=5), encephalopathy (n=3), visual abnormalities (n=3). These neurologic symptoms were cause of PRESS (posterior reversible encephalopathy syndrome) probably due to cyclosporine-A and tacrolimus (n=7), drug toxicity-cyclosporine-A and busulphan (n=8), infections (n=5), intracranial hemorrhage (n=3), hypomagnesemia (n=2), hyperviscosity (n=1). Neurological complications with MFD were seen significantly more than MSD transplantation (p=0.02). Neurological complications were developed significantly more in patients with acute graft versus host disease (aGvHD), grade II-IV aGvHD and cGVHD than without GvHD (respectively p=0.002, p=0.001 and p=0.007). Mortality was significantly more high in patients with neurological complication (p<0.005).
Conclusion: In this study; the frequency of neurological complications was %11.2. We showed that MFD, aGvHD, Grade II-IV aGvHD and cGvHD were significant risk factors to development of neurological complication. Neurological complications were significantly associated high mortality. We concluded that pediatric transplant patients especially received profound immunesuppressive drugs used must be followed closely for development of neurological complications because of high morbidity and mortality rates.

[P1024] **Table 1. The diagnosis of patients developed neurological complications**

| The diagnosis | Number of patients | Percentage of patients |
|---|---|---|
| Thalassemia major | 14 | 51.9 |
| Fanconia anemia | 4 | 14.8 |
| Osteopetrosis | 2 | 7.4 |
| Hemophagocytic Lymphohistiocytosis | 2 | 7.4 |
| Other* | 5 | 18.5 |

*Other: one each patients of amegakaryocytic thrombocytopenia, aplastic anemia, severe combined immune deficiency, leukocyte adhesion deficiency, Diamond-Blackfan anemia

**P1025**

**Cardiac complications after bone marrow transplantation for benign disease in children: single-centre experience from Turkey**

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Pericardial effusion and cardiac complications are rare complications in patients undergoing haematopoietic stem cell transplantations (HSCT). Life-threatening cardiac toxicity is occuring in less than %2 of all patients after HSCT. The incidence of cardiac complications after HSCT in pediatric population is exactly unknown. The objective of this study was to assess the incidence, risk factors and outcome of 240 pediatric HSCT recipients for benign diseases.
Patients and Methods: We retrospectively evaluated 240 pediatric patients who underwent allogeneic HSCT from 1998 to 2011 for the development of post transplant cardiac complications. Cardiac complications occured in 19 patients (7.9%). These cardiac complications are consisted of pericardial effusion (n=9), hypertrophic cardiomyopathy (n=3), reduced left ventricular ejection fraction (n=3), hypertension (n=2) and tacyhycardia (n=2). There was no correlation between overall results of cardiologic evaluation before SCT and cardiac toxicity. Male/female ratio was 12/7. The mean of age was 9.7±6.8 years. Donors were matched unrelated (MUD) in 9, matched sibling donor (MSD) in 7, matched family donor in 3 transplantations. Peripheral blood stem cell was used in 63.2%. All of patients received cyclophosphamide as conditioning regimen. Cardiac complications with MUD were seen significantly more than MSD (p=0.016). Cardiac complications were developed significantly more in patients with aGvHD (p=0.001). In patients developed cardiac complication, mortality rates was significantly higher (p<0.001).
Conclusion: In our pediatric transplant unit, the frequency of cardiac complications was 7.9%. We showed that MUD, aGvHD, Grade II-IV aGvHD were significantly affecting to development of cardiac complication. Cardiac complications were significantly related high mortality. We concluded that pediatric transplant patients especially GvHD group must be followed closely for development cardiac complications. For MUD, the further studies about factors affecting cardiac complication development is needed.

**P1026**

**Chronic granulomatous disease: quality of life and cognitive outcome with conservative treatment or HSCT**

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Background: Chronic Granulomatous Disease (CGD), a rare primary immunodeficiency predisposing to infection and inflammation, is managed with lifelong prophylactic antimicrobials and treatment of complications, or potentially curative HSCT or gene therapy. Chronic illness has an impact on quality of life and mental health. There has been little assessment of this in CGD. Other inflammatory conditions can be associated with cognitive decline. We present preliminary data from quality of life and cognitive assessment of the UK CGD cohort.
Methods: The UK and Ireland CGD registry was compiled in 2000. Most CGD patients are cared for at 3 centres in the UK. Cognitive function was assessed using the Wechsler Abreiviated Scale of Intelligence. Age appropriate standardised questionniares were used to measure emotional, behavioural and self esteem issues and quality of life.
Results: 26 patients (25 male) have been recruited. All have undergone cognitive assessment (median age 13 years, 8 post-HSCT). Mean IQ 95.19 (range 58-130, std 19.6) was not significantly different from the expected value of 100. Mean for those post-HSCT was 93.5 (std 22.6) and 95.94 (std 18.7) for those not transplanted. More patients than expected in a normal population had IQ <85 (30.8%, p=0.032), 2 (7.7%) had IQ <70 indicating severe cognitive difficulties. 10 parents and 7 children (4 post HSCT), have completed Strengths & Difficulties Questionnaires (SDQ). 12 parents and 9 children (5 post HSCT), have completed the Pediatric Quality of Life Scale (PedsQL). Parent reported peer and self reported emotional difficulties were significantly higher in those who had not undergone HSCT compared to the population norm using the SDQ (p=0.009 and p=0.012 respectively). Parent and self reported emotional functioning scores were significantly lower for those that had not undergone HSCT compared to those that had on the PedsQL (p=0.044 and p=0.039 respectively).
Conclusion: Although these are interim results, it is interesting that quality of life appears better following HSCT. The mean

IQ for the population was not significantly different from the norm and there were no differences between the HSCT and non-HSCT groups, but more patients than expected had an IQ of <85 (lower limit of "normal"). Mild cognitive dysfunction may be more common than expected in the UK CGD population. Further work is required to establish whether this is related to intracranial infection, inflammation or other factors.

**P1027**

**Reticular dysgenesis confirmed by homozygous mutations in adenylate kinase 2 gene during uncertainty of granulocyte colony-stimulating factor-related acute myeloid leukaemia**

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Objectives: Reticular dysgenesis (RD) is the most sever form of sever combined immunodeficiency (SCID) that characterized by sever agranulocytosis and impairment of T- and B-lymphocytes. During uncertainty of granulocyte colony-stimulating factor (GCSF)-related acute myeloid leukemia (AML), we confirmed RD form of SCID by mutations in adenylate kinase 2 (AK2) gene thereafter successfully treated with Bone marrow transplantation (BMT).
Method: 4-month-old infant had persistent neutopenia and recurrent sepsis (documented: staphylococcus epidermidis). Granulocyte colony-stimulating factor (GCSF) was used, but without improvement of the neutrophil counts. Repeatedly bone marrow biopsy showed 30-50% blasts cells consistent with AML. The cells were positive for CD13, CD34, CD117, CD33 and HLADR and were negative for CD3, CD4, CD5, CD61, CD8, CD10, CD19, CD14 , CD20, cyCD79a, MPO, CD15 and TDT markers. Full immunologic evaluation was performed.
Result: Complete blood count showed persistent sever neutropenia (leucocytes 600 cells/µl, neutrophils 60 cells/µl, monocytes 40 cells/µl, lymphocytes 480 cells/µl, haemoglobin 10.7 g/dl, and thrombocytes 410,000 cells/µl). The immunological work up showed panhypogammaglobulinaemia (IgA <0.05 g/l, IgG <1.08 g/l and IgM <0.05 g/l ) and sever panlymphopenia (CD3+ 150 cells/µl, CD3+/CD4+ 50 cells/µl, CD3+/CD8+ 100 cells/µl, CD3-/CD19+ 280 cells/µl and CD3-/CD16+/CD56+ 60 cells/µl ). T-cell receptor excision circles (TRECs) using real-time quantitative polymerase chain reaction on DNA showed undetectable TREC circties. Homozygous mutations in the gene encoding the mitochondrial energy metabolism enzyme AK2 confirmed RD form of SCID. Fluorescence in situ hybridization study showed no evidence of leukemic gene rearrangement. He had BMT with successful myeloid and lymphoid engraftments.
Conclusion: GCSF has no proven benefits for RD patients, but can cause malignant transformation. The price mechanism behind the appearance of malignant clone of cells when sever congenital neutropenia treated with GCSF is not yet clearly defined. It is critical to differentiate RD form of SCID from other causes of leukopenias in neonates, because RD is a lethal condition and rapidly fatal disorder. To achieve the best success in such cases, patient with RD should receive bone marrow transplantation treatment as curative therapy of choice.

**P1028**

**Well-being of physicians who work in oncology unit and in BMT unit: analysis of protective factors from work stress**

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Background: Recently a great deal of attention has been given on doctors' work related stress and possibility of improving their quality of life. Several studies report that oncologists are overloaded psychologically. Contact with serious diseases, excessive working pressure, structural weaknesses, are some of the factors that predispose oncologists to stress. In 2008, at the ASPHO annual meeting, pediatric hematologists/oncologists noted burnout (considered as a result of protracted stress at work) was a significant challenge in their lives. To date, no studies have extensively ruled out on protective factors from work stress among pediatric oncologists.
Aims: Our research investigates the relationship between Work Stress, Work Engagement and Personal Well-being in a sample of doctors working in Italian hospitals. Specifically, the study investigates whether Organizational Support, Self-efficacy Perceived, adaptive Coping styles and specific training on social and relational skills mediate between Work Stress, Personal Well-being and Work Engagement. In addition, it investigates the differences between oncologists, pediatricians and other kind of physician.
Materials and Methods: The research included 176 physicians (M=89; F=83; MS=4) working in Italian healthcare units of oncology and onco-haematology; 15 of them work in pediatric unit. Doctors filled self-report questionnaires to evaluate Work Stress and Coping Strategies (Health Professions Stress and Coping Scale), Personal Well-being (General Health Questionnaire), Work Engagement (Utrecht Work Engagement Scale) and two purpose-built scales to measure the degree of perceived Organizational Support and the level of specific training on social and relational skills.
Result: It was found negative and significant correlations between the scores of the scales that measure the use of adaptive coping strategies and stress levels. Moreover, physicians who obtained higher levels of specific training on social and relational skills reported lower levels of stress. Finally, it was highlighted significant differences between pediatricians and other physicians, especially on the use of adaptive coping strategies.
Conclusions: Our results seem to confirm that well-being of physicians is mediated by typical aspects of the profession, such as social skills in relationship with patients.

**P1029**

**Vascular access for the collection of peripheral blood stem cells in paediatric patients**

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Background: High dose chemotherapy with stem cell rescue has been show to impruve event - free survival for children with solid tumors, relapsed or refactory lymphoma, and high - risk form Ewing's sarcoma. PBSC collections using automated or semiautomated blood cell separator in pediatric patients are much more complicated and risky than in adults. It is difficult to maintain adequate vascular access proving a sufficient blood flow rate because of small size of their blood vessels and a lack of compliance of the child during procedure. The aim of this study is to present vascular access for the collection of PBSC in pediatric patients with evaluation feasibility, efficacy and safety of procedure.
Methods: Since November 2002. to August 2011, 93. children (64 male, and 29 female) underwent 124 autologous PBSC collection. The primary diagnosis in 42. children (45%) was neuroblastoma, Ewing sarcoma in 19 (20%), non-Hodgkin lymphoma in 14 (15%), Hodgkin lymphoma in 9 (10%) other tumors in 8 (7%), and AML in 1 (3%) patient. The median age of the patients was 7 years (range, 7 months-18 years), and the median body weight was 31 kg (range, 7-105). Blood was withdrawn through a temporary catheter inserted into radial artery before leukapheresis in 75 procedures (60%), the femoral artery in 16 (13%), femoral vein in 14 (11%), jugular vein in 13 (10), and

cubital vein in 6 (5%) procedures. The processed blood was returned through a central venous catheter in 61 (49%), temporary catheter inserted into femoral vein in 35 (28%), jugular vein in 16 (13%) and the cubital vein in 12 (10%) cases. Median flow rate was 29 mL/min (range, 11-55) and average leukapheresis time was 276 min (range, 136-303). Catheters were removed 4-5 hours after completion of PBSC harvest. There were no immediate or long term complications. Switching the radial catheter to diferent blood vessels during the collection was necessery in eight (6%) procedures. Two (1.6%) of the stem cells products were infected. Tareget dose of $5x10^6$/kg CD34+ cells was realized in 76 (82%) patients.
Conclusion: It is possible to harvest target dose of CD34+ cells in most of children with malignancy using temporary vascular access.

**P1030**
**High-efficiency collection of stem cells from children with a novel programme on the Spectra Optia apheresis system**
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The collection and reinfusion of peripheral blood stem cells in children is an integral part of many current treatment options. We tested two versions of a newly developed stem cell collection program on the Spectra Optia apheresis system, version 3(V3) and version 5(V5).
53 procedures were carried out (31 patients), 45 procedures were eligible for further analysis, (19 V3; 26 V5). Patient diagnosis included neuroblastoma 10, Ewings sarcoma 7, Brain tumour 7, Hodgkins lymphoma 2, germ cell tumour 1, Juvenile idiopathic arthritis 1 and Wilms tumour 3.
Stem cell mobilization was with GCSF either following chemotherapy or using a steady state mobilization. Plerixafor was used for one mobilization in conjunction with GCSF after prior failed mobilization.
Collection preference was set to maximize collection efficiency of CD34+ cells and to limit cross-cellular contamination. Standard blood analysis and CD34+ counting were done pre-apheresis and on the collected stem cell product. The CD34+ collection efficiency was calculated using pre-CD34+ counts and product CD34+ counts only.
Patients who underwent collections on V3 were smaller than on V5 (17 kg (12-59) *vs* 22kg (9.5-67); p<0.02). More blood needed to be processed using V3 (4.5 litres (2.0-8.7) V3; 3.2 litres (1.9-7.8) V5; p<0.009) already indicating that blood can be processed more efficiently on V5. In addition, collection time was lower on V5 (median 244 min V5 *vs* 265 min V3). Stem cell product parameters and efficiencies are summarized in the table below as median values. Statistically significant improvements, as measured by a Mann-Whitney U test, are indicated by (*), p-value attached.

Conclusion: Comparing two software versions using the Spectra Optia MNC apheresis system, we found that procedure time was significantly shorter and that the CD34+ collection efficiency was significantly higher using version 5. The Optia apheresis system has proven to be a safe and efficient system for collection of stem cells in very small children.

**P1031**
**Stem cell collection with the new Optia™ system in children and young adults**
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Introduction: Harvesting autologous stem cells is meanwhile a routine procedure in pediatric patients suffering from malignancies. Recently the OPTIA™ (Caridian BCT, USA) apheresis system was introduced to the medical community. Data are available for the adult population but not in children. We made the following field evaluation as a part of our in-house validation system to gain data about the performance of this new apheresis device in children and young adults.
Patients and Methods: From January to September 2011 we performed 6 autologous stem cell harvests in 6 patients suffering from various malignant diseases (neuroblastoma, hepatoblastoma, ATRT, PNET, AML and Ewing's sarcoma) with the OPTIA™ system. 1 female, 5 male, median age 8.5 y (min 1–max 20), median bodyweight (bw) 27 kg (10.3–54.5), 3/6 with a bw <20 kg, for all of them the set was primed with blood according to the blood priming procedure provided by the system. Buffy coat collection was activated in one patient manually and in the other runs by the algorithm of the system. In total a median 4 cycles (3–13) were collected. Sample were drawn immediately before and after the run from patients blood and from the apheresis product for blood smear, CD34+ cell count, and leukocyte subpopulations according to our in-house protocol. The collection efficiency of leukocyte subpopulations and the loss of hematocrit and platelets were calculated. Mobilisation was performed according to the treatment protocol of the underlying disease.
Results: The CD34+ cell count before was in median 53/µl (25–270), with 28.7 G/l Leukozytes (7.1–55), and 30% hct (21–36). In all patients a sufficient number of stem cells could have been harvested in one apheresis procedure (yield in median 4.8×10E06 CD34+ cells/kg bw; 2.2–17). The amount of total MNC in the product was 76% (69–97). The collection efficiency for CD34+ cells was 67% (27–72), and for total MNC 58% (24–73). There was a significant loss in platelet counts after the procedure (in median 48%; 32–54). No severe side effects in the patients were observed. Once we have observed a leakage in a set.
Conclusion: The Optia™ system showed a good performance in harvesting autologous stem cells also in a pediatric and adolescent population. The procedure is feasible even in pediatric

**[P1030]**

| | Product Volume (mL) | CD34+ Collection Efficiency (%) | Procedure time (min) | CD34+ dose/kg | Qinlet (ml/min) | Time to process 1 TBV (min) |
|---|---|---|---|---|---|---|
| V3 | 80 ml | 42% | 265 min | $2.6 \times 10^6$ | 17 ml/min | 130 min |
| V5 | 100 ml | 62 % (*) | 244 min (*) | $2.3 \times 10^6$ | 13 ml/min | 118 min |
| p-value | | P=0.0004 | P=0.03 | | | |

patients weighing less than 20 kg bw. There is a notable loss in platelets after the procedure. We therefore recommend to observe the platelet count after the procedure carefully.

**P1032**
**Usage of continuous haemodiafiltration in hepatic veno-occlusive disease**
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Hepatic veno-occlusive disease (VOD) occurs in %10-%50 of patients, and remains one of the commonest and most serious complications after hematopietic stem cell transplantation (HSCT). While mild cases usually resolve spontaneously, severe VOD is associated with a grim prognosis. Defibrotide, has emerged as an effective and safe therapy for patients with severe VOD. Continuous hemodiafiltration support to patients with hepatic failure. There is no any knowledge about the usage of continuous hemodiafiltration in hepatic VOD in the literature. We describe three pediatric patient with severe VOD after HSCT, who were treated with defibrotide plus continuous hemodiafiltration.

**P1033**
**Admission of haemato-oncological paediatric patients in intensitive care unit therapy**
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Introduction: Survival of children treated with chemo and/or radiotherapy is improved over the last decades, but some patients might need to be admitted to intensive care unit (ICU) for the occurrence of life threatening complications. Aims of this study were to report incidence,different causes and risk factors for ICU admission;and 60 days survival after ICU admission by different risk factors.
Patients and Methods: Pediatric patients (0-18) yrs in treatment at the Hemato-Oncology Department of Gaslini Children Research Institute during the period January 1999 October 2010 were eligible, with exclusion of children with brain tumours. Data were collected on age at study entry, gender, underlying disease, type of stem cell transplantation (SCT) (autologous; allogeneic matched related donor, MRD; matched unrelated, familiar not fully matched, alternative donor (AD); and umbilical cord blood transplant, UCB).
Results: 1278 patients were eligible and 54 (4%) were admitted in ICU. Table 1 reports frequency and risk factors analysis for ICU admission. Probability of ICU admission varied by underlying disease (p<0.001) with higher frequency for inborn errors, Fanconi anemia and immunodeficiency (22%). Older children (p=0.016), those treated with SCT (p<0.001) and in particular those who received AD (p<0.001) were some likely associated with ICU admission. Respiratory failure was the cause of ICU admission in 23 patients (43%), septic shock in 8 (15%),severe neurological events in 18 (32%), and severe toxicity chemo/radiotherapy/SCT related in 5 (10%). Within 60 days from ICU admission 27 children died for a overall survival of 50%. Mortality was higher in children with malignancy respect other disease (60% vrs 25%, p=0.017) and in those who needed mechanical intubation (p<0.001), while gender, type of diagnosis, age at ICU admission (<9 yrs vrs ≥9 yrs), year of ICU admission (<2005 vrs ≥2005), cause of ICU admission, SCT and type of SCT, type of conditioning regimen were not statistically associated with 60 days mortality.
Conclusions: In our experience age at study entry, underlying disease and SCT represent the risk factors for ICU admission. Regarding the cause of admission, respiratory failure was the most frequent but did not influenced the mortality of these patients, while malignancy and mechanical intubation were associated with higher mortality. The effort should be to early recognized patients who need to assisted ventilation and when possible to perform not invasive ventilation in department of belonging.

**[P1033]** Table 1: Risk factors for ICU admission

| | Total | Patients requiring ICU | Patients not requiring ICU | p |
|---|---|---|---|---|
| Gender, n (%) | | | | |
| Male | 720 | 24 (3) | 696 (97) | 0.072 |
| Female | 558 | 30 (5) | 528 (95) | |
| Median age at study entry, yrs (range) | 5 (0-21) | 8 (0-19) | 5 (0-21) | 0.016 |
| Type of diagnosis, n (%), | | | | |
| Leukemia/Lymphoma | 507 | 35 (7) | 472 (93) | |
| Solid tumors | 625 | 3 (1) | 622 (99) | < 0.001 |
| Hystiocitosis/HLH/SAA | 101 | 6 (6) | 95 (94) | |
| Inborn errors, Fanconi anemia,immunodeficiency | 45 | 10 (22) | 35 (78) | |
| SCT, n (%) | | | | |
| No | 842 | 16 (2) | 826 (98) | < 0.001 |
| Yes | 436 | 38 (9) | 398 (91) | |
| Type of SCT, n (%) | | | | |
| AD | 145 | 28 (19) | 117(81) | |
| MRD | 73 | 6 (8) | 67 (92) | < 0.001 |
| Autologous | 194 | 3(1) | 191 (99) | |
| UCB | 24 | 1(4) | 23 (96) | |
| Total | 1278 | 54 (4) | 1224 (96) | |

# Haemoglobinopathy and Inborn Errors of Metabolism

**P1034**

**Long-term life experience after allogeneic haematopoietic stem cell transplantation for thalassaemia: a first original report**

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Allogeneic hematopoietic stem cell transplantation (HSCT) is the mainstay of curative treatment for thalassemia major. None the less, this therapeutic procedure can potentially compromise health related quality of life (HRQoL) and social life. There is generally a lack of evidence-based data on the long term effects of HSCT, when patients are no longer under the care of the transplant center. This is the first report to provide a cross sectional assessment of HRQoL in thalassemia-free patients after a mean of 20 years from HSCT. One hundred and eight Sardinian patients, transplanted from a sibling donor in Pesaro and Cagliari during the eighties and nineties were selected from the Sardinian Regional Government Health Department's Registry. From August to November 2011, an internationally validated HRQoL questionnaire (SF-36) and a demographic/clinical form were sent to patients and their sibling donors. By the end of November 2011, we received fully completed questionnaires from 54 patients (26 females and 28 males) and 41 sibling donors. Mean age at transplantation was 13.9 (currently 37.9 yrs) and 17.9 (currently 41 yrs) for patients and donors, respectively. The medium follow up from HSCT was 20.8 years (range 13.6-29.4). Sixty-seven percent of patients and 80% of donors are currently working. Among patients, 40% are living with a spouse/partner (donors=61%) and 14.8% had 1 or more healthy babies (donors=31%). Sixty-three percent of patients belong to a social network (donors=46%). Acute and chronic graft versus host disease (GVHD) were reported in 29.6% and 18.5% of patients, respectively. An active illness was reported in 74% of patients (hepatic, pulmonary, endocrinologic, infectious) and 54% are still taking drugs. Patients and donors reported similar HRQoL scores in 5 of 8 subscales of the SF-36 questionnaire (role-physical, body pain, general health, role-emotional and mental health). Significantly lower scores were registered among patients for physical functioning, social functioning and vitality. Clinical, socio-demographic and HRQoL data collected 20 years after HSCT show that thalassemia-free patients experience favorable social and working conditions. Most dimensions of HRQoL were comparable to those of the control population of sibling donors. Lower scores in some scales can possibly be explained by current illness and drug therapies. These data provide further insight into the choice of HSCT for a chronic and non life-threatening disease.

**P1035**

**Haemopoietic stem cell transplant failure followed by switch to stable production of foetal haemoglobin**

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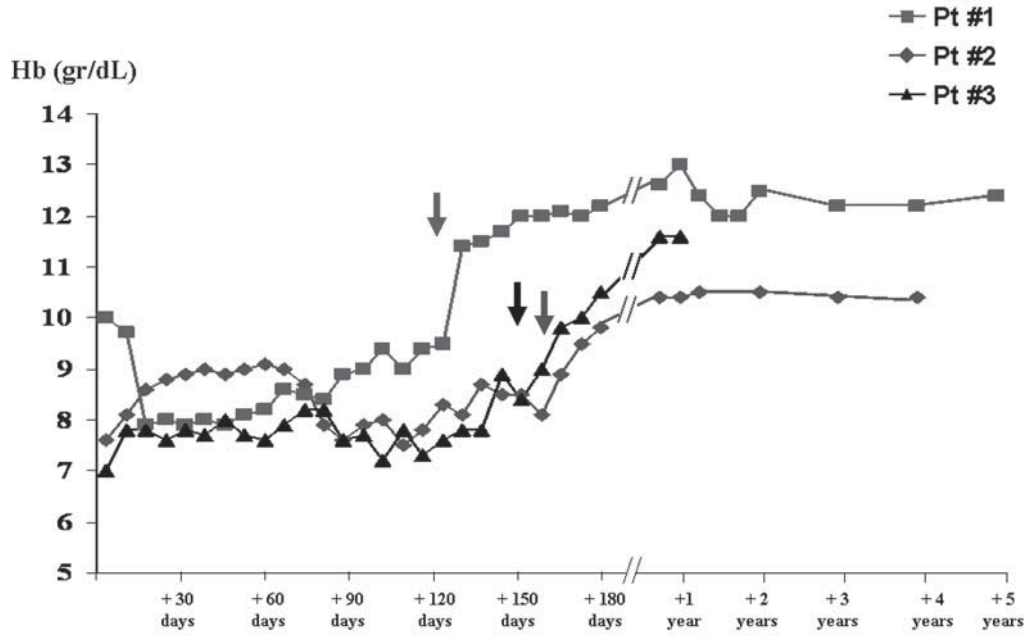
Introduction: Sickle cell anaemia and beta-thalassaemia remain the most common human genetic disorders world-wide. High fetal haemoglobin (HbF) levels are correlated with reduced morbidity and mortality in both diseases. Based on this observation, recent studies provide new insight into the molecular mechanisms of the haemoglobin switching in order to induce the HbF production in adult haemopoietic cells. A strong support to such novel approaches comes from recent clinical

**[P1035]**

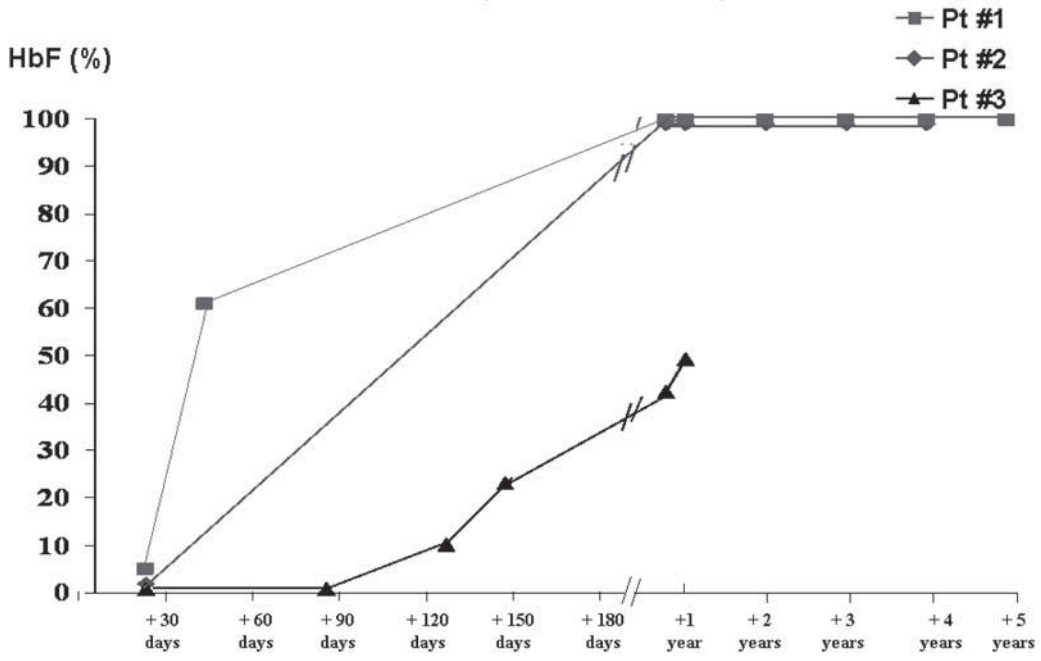
Figure 1 : Total haemoglobin (Hb) levels observed in the study patients from bone marrow transplant until the last follow-up.




The coloured arrows indicate the last transfusion for each patient.

[P1035]

Figure 2: Fetal Haemoglobin (HbF) levels observed in the study patients from bone marrow transplant until the last follow-up.




observations carried out by our group: in three patients affected by beta-haemoglobin disorders the reactivation of HbF synthesis has been documented after bone marrow transplant (BMT) failure and autologous reconstitution.
Patients and Results: At the first, we observed two beta-thalassaemia major cases which were candidates for BMT: the first thalassaemic patient, rejected at +40 days after BMT and the second one at +90 days after transplant. The autologous recovery was documented by the DNA molecular analysis that detected 0% residual donor cells in both cases.
Transfusion therapy was required to support anaemia until +118 and +162 days after transplant in the first and in the second case respectively. Afterwards the Hb levels were steadily over 11.8 and 10.2 g/dl respectively without the use of transfusion support and the Hb electrophoresis revealed HbF 99.8% in both cases.
As we write this at +69 and +57 months respectively of ongoing follow-up after graft failure, both patients maintain the sustained and full (99.8%) production of HbF and are transfusion-free. (See the Figure).
The third case is a sickle cell anaemia patient with pre-transplant HbS level of 77.3%. The patient failed to engraft and the autologous recovery was documented +48 days after the aploidentical stem cell transplant.Twenty months after the BMT failure, the HbF levels increased to 49.2 and the patient remains free of transfusion therapy with stable Hb level over 11.4 gr/dl and without vaso-occlusive symptoms (Figures).
Conclusions: The three cases show that the reactivation of HbF synthesis can occur in the adult age and the high levels of HbF provide a therapeutic benefit to the beta-disorders.
Although the mechanisms underlying the switch back to stable HbF production after BMT failure need further investigation, these cases strongly support the research efforts to reverse the haemoglobin switch and induce the HbF production in adults in order to treat the beta-haemoglobin disorders.

**P1036**

**The chance of finding a matched related donor for haematopoietic stem cell transplantation for patients with Thalassaemia in Jordan**

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Purpose: Hematopoietic stem cell transplantation (HSCT) is the only curative option for patients with thalassemia. The best results were observed following matched related HSCT. Information regarding the likelihood of finding suitable matched related donors for patients with thalassemia is limited. The aim of this study was to evaluate this potential in Jordan, which might reflect other developing and Arab countries.
Patients and Methods: Retrospective review of medical records of all patients with Thalassemia and their families who did HLA typing study at King Hussein Cancer Center (KHCC) in Amman (which has the only comprehensive bone marrow transplantation program in Jordan), between January 2003 and November 2011 was performed.
Results: A total of 341 patients were included, 55% (n=185) were males, and 45% (n=156) were females. The median patient age was 9 year (0.3-32). The median family size was 4, with a range of 1 to 10.
The probability of finding a matched related donor from immediate and extended families was 61% (n=208) for all patients, among them; 13.5% (n=28) were found to have non-siblings related donors from extended family search. Among those patients with donors, 46 patients received HSCT at KHCC, with 98% overall survival and 88% thalassemia free survival.
The average number of donors was 1.3 (with a range of 1-4) for patients who had sibling donors, and 1 (1-2) for patients with non-sibling related donors.
Conclusion: The chance of finding a matched related donor for HSCT for patients with thalassemia is high as compared to Western countries and Asia. We expect to have a similar trend in other developing and Arab countries. A considerable

number of matched donors can be found through extended family search. This might affects the direction of searching for a suitable donor in our area, as we recommend looking for potential extended family donor before unrelated donor search. This might also be applicable for thalassemic patients of Arabic background living in Europe or United States. We also recommend establishing national and regional unrelated donor registries at our area;to provide more possibilities of finding suitable donors to all thalassemic patients in need of successful HSCT.

**P1037**

**First allogeneic haematopoeitic stem cell transplantation for a sickle -ell anaemia patient in Nigeria: a case report**

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Objective: Sickle Cell Anemia has a prevalence of 3% in Nigeria (population of 150 million) and Allogeneic HSCT is an approved curative therapy. We present our first Allogeneic HSCT for a 7 years old patient with severe Sickle Cell Anemia.The patient had a stable Hemoglobin of 7.0g/dl, a previous history of cerebrovascular accident and debilitating right sided hemiparaparesis.
Method: The Patient was HLA identical with his 14 year old sibling. Both patient and donor had an ABO blood group A rhesus positive, were seronegative for CMV. No malaria parasitemia, no evidence of tuberculosis and HIV I and II infection were found. A CT scan of the neurocranum showed few old cerebral micro-infarcts. Conditioning was with (Reduced Intensity Conditioning (RIC)). [Fludarabine 150 mg (30 days -4 to -2) and Bulsulphan 400 mg (4x25 mg 6 hly days -5 to -1) and ATGAM 500 mg (days -4 to-2)]. GVHD prophylaxis was with Cyclosporine A (2x50 mg daily) And Mycophenolate Mofetil (2×500 mg/day). Stem cell source was unmanipulated bone marrow harvested on the 28th of September 2011 with $9.8\times10^6$ nucleated cells per kg body weight in a total product volume of 900mls. Infectious disease prophylaxis was with Bactrim, Variconazole, Acyclovir and Proguanil.
Result: Neutrophil engraftment was day 18 and platelet engraftment day 21. At day 32, chimerism was 36% and hemoglobin electrophoresis since day 12 has been HbAA. Day 70 full blood count was a total white blood cell count of 3100/µl, Neutophils 1200/µl, Hb 11.3 g/dl, Platelet 198,000/µl, and chimerism 69%. Adverse effects were hypertension and prolonged clotting time due to volume overload (over 4 hrs) during the infusion of the stem cells and heparin in the bag whic was managed with Antihypertensive and and protamine sulphate. An exit site Infection of the central femoral Catheter with pseudomonas was the only infectious complication. To date there are no signs of acute or chronic GVHD. Patient had a total of two units of irradiated red cell concentrate and 4 units of platelets concentrate. The Patient was discharged on day 71 and he is currently clinically stable and responding to physiotherapy.
Conclusion: Sickle cell disease is the most prevalent Hemaglobinopathy in Nigeria. With Allogeneic HSCT from a HLA matched sibling we have successfully transplanted a 7 year old Sickle Cell Anaemia. With the assistance of Government and improved Health Insurance Policy we could make HSCT available to many Nigerians who have both malignant and non-malignant disorders.

**P1038**

**The impact of splenectomy on the morbidity and mortality following matched related donor haematopoietic stem cell transplantation for Thalassaemia major patients**

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Splenectomy is a critical decision for thalassemia major (TM) patients because of the blunted primary immune response to encapsulated organisms. Hematopoetic stem cell transplantation (HSCT) produces additional risk for these patients due to intense immunesuppression. A retrospective study was designed to determine the role of pretransplant splenectomy in early and late bacterial infections and survival among 54 patients following first HLA-identical HSCT for TM. Fifteen (27%) of the 54 patients were splenectomized pretransplant. The median follow up time was 65.5 months (12.0-168.3 months).Splenectomized patients were older (14.6±3.0 *vs* 7.7±4.2 yr; p<0.001) and majority of the splenectomized patients were classified as class III high risk group (%86.7 *vs* %13.3; p<0.01). Splenectomized patients had a significantly shorter time to ANC >500/mm$^3$ (12.2±2.0 *vs* 17.2±4.1 days; p<0.001). There were no significant difference in peritransplant infection rate between patient groups. The incidence of acute and chronic GVHD, SOS were similar for splenectomized and nonsplenectomized patients. Based on Kaplan-Meier analyses, patients with splenectomy had a significantly higher overall mortality (five year OS rate: 78.3±1.1 *vs*. 97.4±0.2%). On a multivariate analysis older age and infection had a significant impact on OS. During the peri-transplant period (<100 days) one patient in non-splenectomized group and one patient in splenectomized group died due to infection and acute GVHD, respectively. During long term follow-up period two additional patients died because of sepsis within 13 months after HSCT and both of these patients were in the splenectomized group. In summary, our study showed no significant influence of splenectomy on early posttransplant infections and mortality rate probably because early intervention is possible during early post HSCT period. Unfortunately, during the long term follow-up period risk of infection increases significantly because of additive effects of immunosuppression to the unfavorable effect of splenectomy and it is not possible to achieve early intervention after hospital discharge in a developing country. For this reason the decision of splenectomy should be considered carefully for patients with benign diseases like TM who will undergo transplantation. Prevention of splenomegaly with regular transfusion program should be the main purpose of treatment of the patients with TM.

**P1039**

**Value of transient elastography (FibroScan) in assessment of liver fibrosis in patients with Beta-thalassaemia major considered as candidates for haematopoietic stem cell transplantation**

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Objectives: To assess the diagnostic accuracy of TE in patients with Beta-thalassemia major (TM) who are candidates for hematopoietic stem cell transplantation.
Methods: We prospectively enrolled 58 patients with Beta-thalassemia major (median age: 8 years; range: 2.6-20 years; 65.5% male) in this study. In each patient, we performed liver stiffness measurement using TE expressed in kilopascals (kPa) and liver biopsy assessed according to the Ishak score. The diagnostic value of TE was compared with the histological fibrosis stage using linear discriminant analysis (the area under the receiver operating characteristic curves (AUROCs)).
Results: Twenty six patients (44.8%) had mild fibrosis, 12 patients (20.7%) had moderate fibrosis and 2 (3.4%) patients had severe fibrosis. TE values were significantly correlated with thalassemia classification (p<0.001), iron deposition (p<0.037) and fibrosis stage (p=0.008). Median TE values in patients with severe fibrosis (stage 3-5) and mild or no fibrosis were 4.5 (range, 3.0-13.0) kPa and 4.0 (range, 2.5-9.0) kPa, respectively. For predicting high fibrosis stages (stage ≥ 3), with cut-off of 4.35 kPa, AUROC was 0.670 (95% confidence interval [CI]: 0.508-0.833) with 76.9% sensitivity (95% CI: 70.8-81.8) and 57.8% specificity (95% CI: 53.3-60.3).

Conclusion: TE appears to be an accurate method for the diagnosis of fibrosis stage in patients with Beta-thalassemia major who are candidates for hematopoietic stem cell transplantation .It can also be used as a valuable tool to follow-up the liver status in patients with Beta-thalassemia major after transplantation.

**P1040**

**Successful unrelated bone marrow transplantation in two siblings with alpha-mannosidosis**

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Alpha-mannosidosis is an autosomal recessive inherited lysosomal storage disorder characterized by immune deficiency, facial and skeletal abnormalities, hearing impairment and mental retardation. Although HSCT is considered the only clinically available approach to enzyme replacement, efficacy and safety data are very limited.We report two siblings who successfully underwent HSCT from MUDs.
Case 1: The girl first came to clinical attention due to delay in speech and pectus carinatum at age of 8 y old. She was diagnosed as Alpha-mannosidosis by absent of Alpha-mannosidase enzyme activity in the leukocytes. She had coarse facial features with frontal bossing, skeletal abnormalities. Last 3 years she was suffering from hearing impairment, swallowing difficulty and weakness in the legs. At the age of 11 years old, she underwent BMT from a HLA-C allel MMUD. The conditioning regimen was consisted of Bu+CY and ATG . She received CsA and MTX for GVHD prophylaxis. The number of TNC was $4x10^8$/kg and CD34+ cells number was $1,8x10^6$/kg CD34+ cells/kg, recipient weight. She achieved successful neutrophil and platelet engrafment on day 20. After 2 years, she is now well, swallowing function is improved and put on weight, her communication skills get better. In the last visit, chimerism analysis is revealed 100% of donor cells and her enzyme activity is normal.
Case 2: A 6 y old boy was admitted to the hospital because of skeletal deformity and the family history of Alpha-mannosidosis. He diagnosed as Alpha-mannosidosis by absent Alpha-mannosidase enzyme activity in the leukocytes. He had not expressive speech and was using hearing aid. During following examination, strabismus was determined. At 8 years old, he underwent BMT from 10/10 MUD. He received BU+CY and ATG as conditioning regimen. CsA and MTX were used as GVHD prophylaxis. The number of infused TNC was $13,6x10^8$/kg and CD34+cells number was $10,6x10^6$/kg. He achieved successful neutrophil and platelet engraftment on day 14 and 10 respectively. At 6th month examination, communication skills get better, his coarse facial features start to improve. The last chimerism analyses showed 100% of donor cells and his enzyme level is normal. In conclusion, HSCT should be considered as a therapeutic approach in patients with alpha mannosidosis. The benefits are greater in younger patients before disease related complications have developed. MUDs may use as a stem cell source if MFDs are not available.

**P1041**

**Stem cell transplantation for haemophagocytic lymphohistiocytosis in children: experience at two Israeli centres**

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Hemophagocytic Lymphohistiocytosis (HLH) is an aggressive disorder of immune regulation that carries a grave prognosis; stem cell transplant (SCT) after induction therapy is the recommended definitive treatment. Unrelated donors (UD) are used for patients (pts) lacking a matched family donor, and for those lacking a molecular diagnosis (as sibling donors might themselves be genetically at risk but not yet affected). Two pediatric centers collectively transplanted 22 pts with HLH between 1991 and 2011. Etiologies included mutations of Perforin (7 pts), Munc 13 (2), Munc 18 (3), Syntaxin (1), as well as T-cell lymphoma (1) and EBV infection (1). No molecular or infectious etiology was identified in two extensively evaluated pts, and treatment predated the availability of molecular diagnosis in 5 pts. Twelve pts were in complete response at the time of transplant and 6 had less than a complete response; response data were unavailable in 4 pts. Unrelated donors were used for 10 children. Grafts were derived from marrow (12), peripheral blood (8), cord blood (1), or cord blood + marrow (1). Six pts, mostly transplanted in the 1990's, received fully ablative conditioning; others received fludarabine containing regimens; 91% received serotherapy during conditioning. Cyclosporine was administered after transplant usually in combination with either methotrexate or mycophenolate mofetil. Fifteen patients (68%) are alive (8/10 UD, 7/12 related donors) without evidence of disease, four of whom have stable mixed donor chimerism of less than 70%. Four of 6 pts with signs of active HLH at the time of SCT survive, as did 4/5 children with HLH involvement of the central nervous system at diagnosis (two without apparent developmental delay). Fatal cases of pneumonia were caused by CMV (1), RSV (2), and adenovirus together with aspergillus (1), suggesting that aggressive anti-viral and anti-fungal prophylaxis is imperative in this high-risk population. The child with lymphoma died of multi-organ failure 1 day after transplant, and an additional pt succumbed to pulmonary veno-occlusive disease 5 months after SCT. Severe graft *vs.* host disease occurred in only one child. SCT from related and unrelated donors using fludarabine-based reduced intensity conditioning with the addition of serotherapy is an effective treatment for HLH.

**P1042**

**Allogeneic matched sibling donor bone marrow transplantation after low-dose busilvex-fludarabine conditioning in a 3 year old boy with ataxia-telangiectasia syndrome and acute lymphoblastic leukaemia: a case report**

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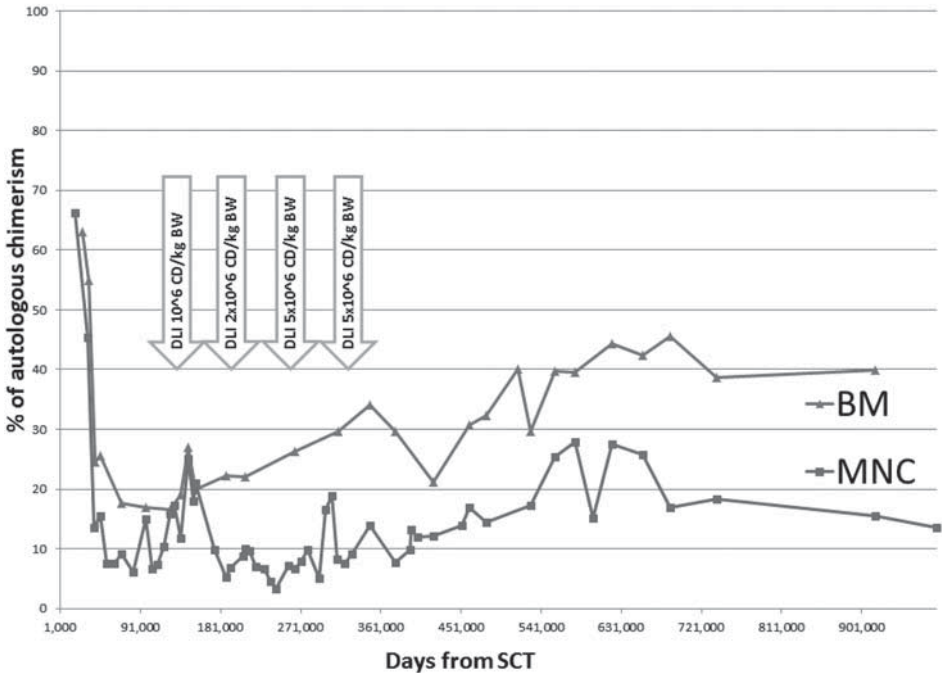
Here we report the matched sibling donor bone marrow transplantation (BMT) in a 3 year old boy with ataxia-telangiectasia syndrome (ATS) and acute T-cell lymphoblastic leukemia. The child was treated for leukemia with an ALL-BFM 2002 protocol, with chemotherapy dosage adjusted for patients with ATS. The indication for BMT was poor prednisone response on the 8. day of induction therapy.
Method: The donor was the HLA-matched brother, in whom ATS was excluded. Conditioning regimen consisted of a protocol used in Fanconi Anemia (GEFA): intravenous Busilvex 2x1 mg/kg body weight (BW), Fludarabine 5x30 mg/m$^2$, ATG-Fresenius 3x20 mg/kg BW. Graft material was bone marrow (BM) containing 3,64 x$10^6$ CD34+/kg recipient BW. GVHD prophylaxis consisted of cyclosporine A, methylprednisolone and OKT-3 (from day +1 to +20).
Results: The chemotherapy induced toxicities after BMT were leukopenia (0.25 k/uL) with agranulocytosis (0.13 k/uL) and mucositis (grade II). Immunosuppression induced multiple viral infections with adenovirus, BKV hemorrhagic cystitis, and EBV-LPD, which course was relatively mild and were successfully treated with cidofovir and rituximab. The ANC recovery was achieved on day +15, with a mixed chimerism (MC) with 66% of cells of recipient origin. The patient received 4 donor lymphocyte infusions (DLI's) for persistent and increasing MC.

**[P1042]** **Chimerism after matched sibling BMT in a child with Ataxia-Telangiectasia syndrome**




The DLI's were given in months +4, +5, +8, +10 after BMT, the T-cell dose was incrementally increased from 1 to 50x$10^6$ CD3+ cells/kg BW. During observation the autologous chimerism in CD3+ subpopulation was in the range of 1.7-5.6%, whereas both in BM and in peripheral blood (PB) the MC reached higher values with initially increasing, but now decreasing trend. Further DLI's and attempts for second BMT procedure were refused by the patient's parent. Despite MC, the patient remains 3 years after BMT in complete hematological remission with normal complete blood count values. The transplantation did not worsen the patient's neurological status.

Conclusions: The GEFA protocol was earlier used in our centre in patients with Fanconi Anemia, and in 1 child with Nijmegen Breakage Syndrome. The patient with ATS recovered after conditioning regimen with mild toxicities, confirming that BMT in patients with malignancy and ATS remains a feasible and promising option. Low hematological toxicities and MC in our patient suggest that ATS patients could be conditioned more intensively than other chromosomal-breakage syndrome patients.

**P1043**

**Reduced-intensity haematopoietic stem cell transplant rescues immune function and corrects pulmonary alveolar proteinosis in DCML deficiency/GATA 2 mutation**

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The novel syndrome of Dendritic Cell, Monocyte, B and NK lymphocyte (DCML) deficiency has recently been characterised and found to be caused by mutations in the transcription factor GATA-2. We describe a series of 10 patients and show that there is a high risk of death unless haematopoietic stem cell transplantation (HSCT) is performed.

We describe 10 patients; 4 sporadic cases and 6 cases from 2 pedigrees showing autosomal dominant inheritance. 7 patients died before DCML deficiency was recognised. Of the 3 patients currently alive, 2 were transplanted and show marked resolution of their disease with a follow up of 36 and 11 months, respectively. Causes of death in the patients who died include disseminated mycobacterium avium infection (2) H1N1 influenza (1) candidiasis (1) pulmonary alveolar proteinosis (2) and CMV pneumonitis (1).

All patients had monocytopenia and decreased B and NK cells with normal T cells at presentation. Haemoglobin, platelets and neutrophil counts were within normal ranges or mildly reduced. In 4 patients, near absolute DC deficiency and elevated Flt-3 ligand were also confirmed. BM aspirate showed dysplastic megakaryocytes and increased fibrosis in some instances. GATA-2 mutation was confirmed by Sanger sequencing in all cases.

Two patients underwent reduced intensity allogeneic HSCT with PBMC from unrelated adult donors (patient 1-10/12 HLA match; patient 2-9/12); transplant conditioning was with Fludarabine 150 mg/$m^2$, Melphalan 140 mg/$m^2$ and Alemtuzumab 60 mg (patient 1), and Fludarabine 150 mg/$m^2$, Busulphan 6.4 mg/kg, Alemtuzumab 60 mg (patient 2). GVHD prophylaxis was with Ciclosporin and Mycophenolate Mofetil. Both transplants were uneventful.

Patient 1 is now 32 months post-transplantation, well and off all medication. The DC count, monocyte count, lymphocyte subsets and immunoglobulins are normal and there were good responses to tetanus and HIB vaccinations. Patient 2 is now 9 months post-transplantation with significant improvement in respiratory function and no longer requiring oxygen. DCs and monocytes are in the normal range. Myeloid chimerism is 100% and T cell chimerism >85% in both. Lung function tests have improved to within 90% of predicted normal and radiology shows almost complete clearing of pulmonary infiltrates. Follow-up continues with gynaecology for VIN3 associated with HPV16/18 infection. Neither patient has developed GVHD.

**P1044**

**Haematopoietic stem cell transplantation for infantile malignant osteopetrosis – a single-centre experience**

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Introduction: Hematopoietic stem cell transplantation (HSCT) is currently the only curative treatment for infantile malignant osteopetrosis (OP). Experience with HSCT is available but limited, and outcome data is needed to plan the best treatment regimen.
Aim: We report data on 28 patients with a severe OP phenotype who underwent HSCT in our center from 1983 to 2011.
Methods: 22 boys and 6 girls (aged 1 month to 3 years, median 6 months), who underwent 29 allogeneic HSCTs were included in this retrospective study. In the first twelve patients (group 1) transplanted prior to 1996 the conditioning regimens included busulfan (Bu) 16 mg/kg with cyclophosphamide (Cy) 200 mg/kg in 5 patients, in four patients Bu 16 mg/kg + Cy 200 mg/kg was augmented with thiotepa, two additional children were conditioned with a combination of total body irradiation and Cy and one more patient received Bu-mitoxantron-Cy. In four of these 12 patients alemtuzumab was added to the conditioning regimen. Sixteen children transplanted after 1998 (group 2) received a fludarabine-based regimen. Nine patients were conditioned with fludarabine-busulfex and in five children thiotepa was added to the fludarabine-busulfex backbone. Two patients who underwent haploidentical transplantation were prepared with fludarabine-busulfex-melphalan and treosulfan-cyclophosphamide, respectively. All but two children from this group received additional serotherapy (ATG). In the first group, donors included 10 matched siblings, 1 matched family and one mismatched family donor. In the second group 10 donors were full matched siblings, four matched family donors and two family mismatched donors. Follow up through November 2011 ranged from 2 to 280 months.
Results: In group 1, 5 patients (42%) are alive with a median follow up of 216 months. All of them survive with complete clinical, immunologic and hematologic recovery.
In group 2, all children survive, with 15/16 showing stable long term engraftment. One child who underwent a haploidentical transplantation demonstrated graft loss and was successfully retransplanted. Three of five surviving patients in group 1 developed cGvHD compared with none in group 2.
Conclusion: HSCT in children with OP is associated with a very favorable outcome after fludarabine–based reduced intensity conditioning. Further studies to determine the best conditioning regimen and optimal alternative donor selection are required.

**P1045**

**NIH-defined chronic graft-versus-host disease and graft-versus-leukaemia effects in patients receiving allogeneic haematopoietic cell transplantation for acute lymphoblastic leukaemia**

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Objective: In 2005 a NIH consensus conference has introduced precise diagnostic criteria and a new severity score for chronic graft-versus-host disease (cGVHD). The new criteria are well accepted by the community but data regarding their prognostic value in specific indications remains limited. We therefore retrospectively analyzed NIH-defined cGVHD in 147 consecutive acute lymphoblastic leukemia (ALL) patients who received a first myeloablative transplant at our center between 1995 and 2009. A subgroup analysis was performed in patients who received pre-emptive donor lymphocyte infusions (DLI).
Methods: Median age was 31 years. Disease status was CR1 (50%), CR>1 (22%) or no CR (28%). Conditioning consisted of 12 Gy TBI ± etoposide ± cyclophosphamide. Donors were HLA-matched related (42%), -matched unrelated (46%) or -mismatched (12%) and peripheral blood stem cells (74%) or bone marrow (26%) were given. GVHD-prophylaxis consisted of CSA/MTX (72%), CSA/prednisolone (19%) or other CSA-based regimens (9%). 44 patients with mixed chimerism or minimal residual disease without GVHD after cessation of immunosuppression received DLI at a median of 138 days.
Results: Median follow-up was 60 months. Projected overall survival (OS) at 1, 2 and 5 years was 61%, 52% and 43%. 5-year cumulative incidence of relapse was 33% and of non-relapse mortality (NRM) 26%. Median time until onset of cGVHD was 121 days (ranger: 24-464) after transplant and 92 days (14-294) after DLI (P=NS). The cumulative incidence of cGVHD was 36% after transplant (mild 7%, moderate 11%, severe 18%) and 58% in patients who received DLI (mild 21%, moderate 14%, severe 23%). cGVHD was subclassified as overlap syndrome *vs.* classic cGVHD in 62% *vs.* 38% of transplant cases and 56% *vs.* 44% of DLI cases (P=NS). Organ involvement was similar for transplant and DLI, with the exception of GVHD of the lung which was not observed after DLI. In multivariate Cox regression analysis with GVHD as time-dependent covariate cGVHD was associated with superior OS which was due to lower relapse incidence (Table 1). Classic and overlap cGVHD

**[P1045]**

| | OS | | Relapse | | NRM | |
|---|---|---|---|---|---|---|
| | HR | P | HR | P | HR | P |
| **cGVHD All patients** | | | | | | |
| None | 1 | | 1 | | 1 | |
| Mild | 0.54 (0.21-1.36) | 0.19 | 0.52 (0.13-2.04) | 0.35 | 0.69 (0.18-2.66) | 0.59 |
| Moderate | 0.18 (0.05-0.60) | 0.005 | 0.34 (0.10-1.13) | 0.078 | 0.18 (0.02-1.43) | 0.11 |
| Severe | 0.47 (0.23-0.94) | 0.032 | 0.14 (0.04 -0.44) | 0.001 | 0.69 (0.24-1.98) | 0.49 |
| **cGVHD DLI patients** | | | | | | |
| None | 1 | | 1 | | 1 | |
| Mild | 0.36 (0.11-1.16) | 0.088 | 0.76 (0.18-3.25) | 0.71 | 0.39 (0.041-3.70) | 0.41 |
| Moderate | 0.11 (0.014-0.88) | 0.037 | 0.25 (0.029-2.11) | 0.20 | 0.00 (0.00-) | 0.99 |
| Severe | 0.18 (0.05-0.67) | 0.011 | 0.13 (0.015-1.08) | 0.05 | 0.21 (0.021-2.06) | 0.18 |

**Table 1. Prognostic impact of cGVHD on OS, relapse and NRM.** cGVHD was added as a time-dependant covariate to a multi-variate Cox regression model which included all relevant transplant and patient characteristics. Only results for cGVHD are shown. Results are reported for all patients (n=147) and for the subgroup which had received DLI (n=44).

had no differential prognostic impact. The positive prognostic impact of NIH-defined cGVHD was also seen in the subgroup of patients where cGVHD was induced by DLI.
Conclusions: NIH-defined cGVHD induces a potent anti-tumor effect in patients transplanted for ALL. This appears to be independent of whether cGVHD develops directly after transplant or whether it is induced by DLI.

**P1046**
**Rituximab in treatment of chronic graft-versus-host disease**
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Introduction: The anti-CD20 antibody rituximab (R) is frequently applied for the treatment of chronic graft-versus-host-disease (cGvHD) or transplant-related autoimmune-conditions like immune thrombocytopenia (ITP). We retrospectively evaluated the efficacy of R for the treatment of cGvHD within the Regensburg and Rostock University transplant program.
Methods: 32 allogeneic stem cell recipients with a median age of 47.5 years (range 19-65) receiving R for cGvHD (moderate n=10, severe n=16) or isolated transplant-associated ITP (n=6) at a median of 913 d (range 127-2878) after stem cell transplantation were evaluated with a median follow-up of 912 d (range 36-2152) after R therapy. Cutaneous manifestations were observed in 24 patients and 16 patients suffered from fasciitis. 14 patients (ITP n=4, cGvHD n=10) received R as 2nd-line treatment and 17 patients (ITP n=1, cGvHD n=16) after failure of 2nd-line treatment, while 1 ITP-patient received R as 1st-line treatment. One treatment course consisted of 4 doses of 375mg/m$^2$ applied at weekly intervals. Seven patients received 2 courses of R.
Results: In the ITP group complete response (CR) was achieved in 4 patients, partial response (PR) in one patient, no response in one patient. Four patients received steroids as additional therapy, two R as monotherapy. In the cGvHD group response was evaluable 24 of 26 patients: 4 patients achieved a CR, 8 patients a PR, 7 patients showed a non durable minor response and 6 patients failed R treatment.
Apart from one non-responder all patients received R as part of a multi-agent regimen. All 6 cGVHD-patients receiving a 2nd course of R as part of a multi-agent regimen achieved a PR. Of note, 15 patients (47%) developed severe infectious complications requiring hospitalization at a median of 123 d (range 53-891) after treatment initiation, including 3 patients with intensive care treatment. Six patients died between day 36 and 567 after their 1st R course, from infectious complications (n=5) or pulmonary embolism (n=1).
Conclusions: R as part of a multi-agent therapy was associated with a significant response rate in ITP and to a lesser extent in cGvHD. Patients with severe cGVHD suffer from immunodeficiency per se and the high frequency of infectious complications after R therapy suggests that it should be reserved for patients failing 2nd-line treatment and requires monitoring for opportunistic infections and intensified anti-infectious prophylaxis should be considered.

**P1047**
**A scoring system predictive of extensive chronic graft-versus-host disease after allogeneic stem cell transplantation**
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cGVHD is one of the most serious consequences of allo-SCT associated with high morbidity and mortality. Identification of potential predictors of cGVHD is crucial. The aim of this study was to expand the search for cGVHD biomarkers, to validate candidate proteins using high-throughput assays in a series of 152 consecutive patients treated in a single center, and to determine a composite prognostic score for prediction of extensive cGVHD.
Patients included in the analysis were treated between 2005 and 2008 and had a median follow-up of 2.3 y (Table 1). In this series, 70 patients were diagnosed with cGVHD (23 with a limited form and 47 with an extensive form) after a median of 7.14 months after allo-SCT. At 2 years, the cumulative incidence of extensive cGVHD was 40% and overall survival of the whole cohort was 69%. Serum samples were collected around day 100 (range, 83-119) after allo-SCT. Forty-one cytokines were studied using Luminex xMAP technology.
In multivariate analysis, 2 clinical factors were associated with extensive cGVHD occurrence: history of prior acute GVHD (P=0.001) and the use of PBSCs as graft source (P=0.006). Ten cytokines were found to be significantly correlated with the incidence of extensive cGVHD. High levels of IP10 (p=0.001), IL15 (p=0.028), IL10 (p=0.011), IL2RA (p=0.026) and MIP-1beta (p=0.010) were associated with higher incidence of extensive cGVHD, while high levels of Fractalkine (p=0.008), MDC (p=0.001), RANTES (p=0,008), TARC(p=0.004), IL12p40 (p=0.015) were associated with a lower risk of developing extensive cGVHD.
Then, we established a practical prognostic score that was calculated using the multivariate Cox model combined with the statistical approach called “time-dependent ROC curves”. We have focused on the 2 significant clinical variables and the most significant cytokines found in order to obtain a useful tool in the daily medical practice. Based on 0,632 bootstrap resampling method for repeated cross-validation, the AUC was 0.80 (95%CI, 0.72-0.87) indicating that such composite score is a powerful predictor of the risk of extensive cGVHD at 2 years.
In summary, results from this study allowed to build a new noninvasive score to accurately predict the risk of extensive cGVHD occurrence after allo-SCT. Such score could be used as a decision tool in the clinical management of allo-SCT. We are currently undertaking an additional validation of this score on another independent cohort.

[P1047]

| | N=152 |
|---|---|
| Median age | 49 (17-70) |
| Gender of the patient (M/F) | 83 (55%) / 69(45%) |
| Diagnosis: | |
| Acute leukemia | 58 (38%) |
| Lymphoma | 45 (30%) |
| Chronic leukemia | 16 (10%) |
| MDS/MPS | 17 (11%) |
| Plasma cell disorder | 12 (8%) |
| Bone marrow failure including AA | 4 (3%) |
| Stem-cell source: | |
| BM | 28 (18%) |
| PBSC | 108 (72%) |
| Cord blood cells | 18 (12%) |
| Donor type: | |
| HLA id. sibl | 70 (53%) |
| MUD (10/10) | 60 (40%) |
| Mismatched unrelated donor | 22 (14%) |
| Conditioning: MAC/ RIC | 48 (32%) / 104 (68%) |
| Acute GVHD | 67 (44%) |
| Grade II-IV | 49 (32%) |
| Grade III-IV | 22 (14%) |
| Chronic GVHD | 70 (46%) |
| Localized/Limited | 23 (15%) |
| Extensive | 47 (31%) |

Table 1: characteristics of the population

**P1048**

**Incidence of graft-versus-host disease and the factors for development of graft-versus-host disease: single-centre experience**

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Introduction: Graft Versus Host Disease (GvHD) is the most frequent complication after allogeneic haematopoietic stem cell transplantation (AHSCT). GvHD can occur despite agressive immunosuppressive prophylaxis even when the donor is a HLA identical sibling.

Patient and Methods: A total of 50 patients who underwent AHCST between July 2010-November 2010 were investigated, retrospectively. All transplantation procedures were performed from fullmatch donor and Seattle regimen was used for GvHD prevention. The frequency of acute GVHD (aGVHD) and chronic GvHD (cGVHD) and factors related with development of GvHD including recipient age, donor age, recipient gender, donor gender, recipient-donor gender match, blood group compatibility, amount of CD34+ given and conditioning regimen were investigated.

Results: The diagnosis of the patients were as follows: acute leukemia (AL) in 36 patients (%72), aplastic anemia (AA) in 5 (%10), myelofibrosis in 3 (%6), non-Hodgkin lymphoma (NHL) in 3 (%6), other diseases in 3 (%6) (Hodgkin lyphoma, sickle cell anemia and myelodysplastic syndrome). 21 of patients were female (%42) and 29 patients were male (%58). 30 (%60) of patients were given non containing total body irradiation (TBI) conditioning regimen, 20 (%40) of patients were given total body irradiation (TBI). The median age of 50 patients was found 30.5 years (min-max: 21-41). The incidence of aGvHD was 26% (13 patient) and cGvHD was %20 (10 patient). No statistically significant difference was determined among development of aGvHD and diagnosis, recipient age, donor age, recipient gender, donor gender, recipient-donor gender match, blood group compatibility, amount of CD34+ given and conditioning regimen. Statistically significant difference was determined between increase in patient age and development of cGvHD (p=0,016). 6 of 12 patients with chronic GvHD between women who have female donor, 6 between no GvHD, donor male in any of the 9 patients with no chronic GvHD. According to the state of women's chronic GvHD was stastically significant difference between the donor's gender (p=0,012).

Conclusion: Increasing patient age and transplantation performed between female recipient-female donor increases risk of cGvHD development.

**P1049**

**Correlation between severity of chronic graft-versus-host disease and allogenic haematopoietic stem cell transplantation outcome: a retrospective monocentre study based on NIH classification**

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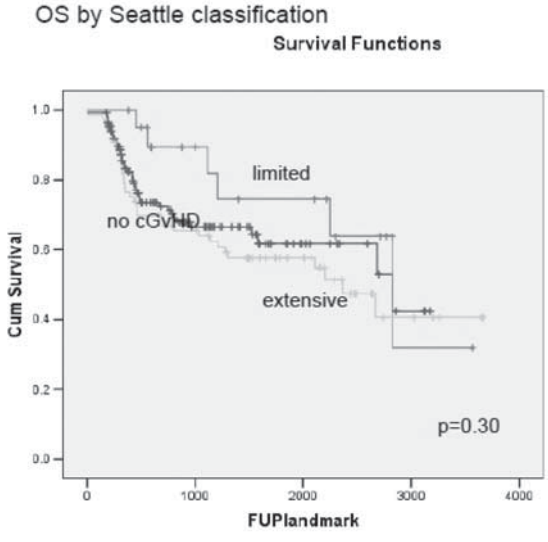
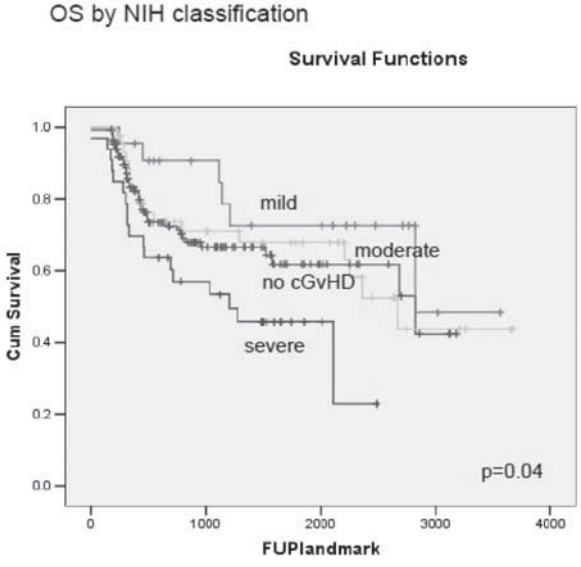
Background: Severity of chronic graft versus host disease (CGVHD) after allogeneic hematopoietic stem cell transplantation (HSCT) is strongly related with outcome. Several staging systems have been developed. Revised Seattle classification stratifies CGVHD into limited or extensive form. In 2005, the NIH published consensus criteria for diagnosis, organ scoring and global assessment of CGVHD severity (mild, moderate or severe). We retrospectively reclassified a cohort of patients receiving reduced-intensity conditioning (RIC) HSCT, using both Seattle and NIH classification and determined the impact of cGVHD severity on the outcome.
Patients and Methods: We evaluated all adult patients with hematological lymphoid or myeloid malignancies who received HSCT from related or unrelated donor, using peripheral blood stem cells, after RIC regimen (fludarabine-busulfan-ATG) between 1998 and 2010 at the Institut Paoli-Calmettes (Marseille, France). CGVHD was classified with both classifications, and was correlated with overall survival (OS), non relapse mortality (NRM), and relapse. CGVHD was considered as time-dependent variable, and included in uni- and multivariate models, after adjusting for age, disease risk, HLA compatibility, graft source and comorbidity score. Relapse or death before CGVHD was considered as a competing event.
Results: 318 patients were evaluated, 130 developped CGVHD (27 limited, 95 extensive forms). Median follow up was 607 days, median age was 50, transplanted for acute leukemia (120), lymphoma (79), multiple myeloma (49), MDS (22), CLL (12), CML (17) or others (19). Peripheral stem cells were mostly used (297 versus 21 bone marrow), from related (228) and unrelated (90) donors. There were 52 de novo, 43 quiescent and 30 progressive forms. Using NIH criteria, we got 28 mild, 52 moderate and 41 severe forms. 22 of 27 limited forms were reclassified as mild, extensive forms were divided into 49 moderate and 39 severe forms. In multivariate analysis, mild and moderate forms were associated with better OS. Severe cGVHD was associated with significant increase in NRM. Only age was statistically significative in OS and NRM models.
Conclusion: Mild to moderate CGVHD have a better OS than patients without or with severe cGVHD, due to lower NRM than patients with severe CGVHD and at least a comparable antitumoral effect with respect to patients without CGVHD. This invites developing strategies limiting severity of CGVHD but not abrogating it.

**[P1049]**

Multivariate time-dependant Cox regression analysis

| | OS | | | NRM | | | Relapse | | |
|---|---|---|---|---|---|---|---|---|---|
| | HR | 95% IC | p | HR | 95% IC | p | HR | 95% IC | p |
| No cGVHD (N= 162) | 1.00 | | | 1.00 | | | 1.00 | | |
| Mild (N=28) | 0.42 | 0.19 - 0.93 | 0.03 | 1.23 | 0.42 - 3.56 | 0.71 | 0.70 | 0.19 - 2.53 | 0.59 |
| Moderate (N=52) | 0.49 | 0.26 - 0.93 | 0.03 | 1.49 | 0.63 - 3.56 | 0.37 | 0.48 | 0.13 - 1.71 | 0.26 |
| Severe (N=41) | 1.14 | 0.66 - 1.98 | 0.64 | 3.18 | 1.45 - 6.95 | 0.004 | 0.53 | 0.15 - 1.87 | 0.32 |



**P1050**

**Chronic graft-versus-host disease after allogeneic stem cell transplantation: a retrospective analysis of risks factors and outcome**

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Chronic graft-versus-host disease (GvHD) still remains one major complication after allogeneic stem cell transplantation resulting in high morbidity and mortality. We retrospectively analyzed 201 patients (median age 42 years) with chronic GvHD at the Ludwig-Maximilians-University hospital munich following hematopoietic cell transplantation between 1997 and 2007 according to the National Institutes of Health (NIH) consensus criteria on diagnosis and staging of chronic GvHD.
All patients underwent first allogeneic stem cell transplantation: 76 from an HLA identical sibling donor, 9 from an HLA haploidentical donor, 99 from an HLA compatible unrelated donor and 17 from an HLA mismatched unrelated donor.
115 patients (57%) developed de novo or quiescent chronic GvHD, 86 patients (43%) progressive GvHD. The overall survival was significantly different with 69% after 1 year, 61% after 2 years, 47% after 5 years and 43% after 10 years for the progressive group compared to 88% (1 year), 75% (2 years), 60% (5 years) and 52% (10 years) for patients with de novo or quiescent chronic GvHD (p=0,04). Thrombocytopenia as one important risk factor for the outcome of chronic GvHD showed significantly worse overall survival after 1, 2, 5 and 10 years with 65%, 49%, 37% and 37% for thrombocytopenic patients (platelet count below 100.000 per microliter) in contrast to 94%, 88%, 72% and 60% for the other group (p<0,001).
Defined by the NIH consensus criteria 74 patients (37%) suffered from classic chronic GvHD and 127 (63%) developed an overlap syndrome. The survival rates were 96% for classic chronic GvHD versus 70% for overlap patients after 1 year, 87% versus 59% after 2 years, 64% versus 48% and 49% versus 47% after ten years. Thus the overlap syndrome significantly impaired the probability of survival after 1, 2 and 5 years whereas the survival rates after 10 years were nearly similar.
In conclusion the onset of chronic GvHD, thrombocytopenia and type of chronic GvHD were confirmed as prognostic factors for survival in chronic GvHD patients after allogeneic stem cell transplantation.

**P1051**

**Response to immunosuppression and severity of chronic graft-versus-host disease at 12 months post-transplant predicts outcome among patients with haematological malignancies**

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Introduction: Chronic graft-versus-host disease (cGvHD) is known to be associated with lower relapse risk, due to graft-versus-tumor effect. Extensive form of cGvHD is associated with high transplant-related mortality (TRM), mainly due to infectious complications as a consequence of the immunosuppressive treatment (IS). Data on main registries and publications deal with maximum grade of cGvHD and its day of presentation post-allogeneic stem cell transplantation (HSCT); however, they did not include information on evolution under treatment and duration of IS delivered.
Patients and Methods: study population was represented by adult patients receiving HSCT after fludarabine-busulfan-ATG conditioning between May 1999 and June 2010 at out Institution for a hematological malignancy. cGvHD was retrospectively classified according to NIH criteria and presentation.

**[P1051]**

| At 3 months | At 6 months | At 12 months | GROUP | n= |
|---|---|---|---|---|
| Never cGvHD, at any time | | | 0 | 183 |
| | | | | |
| Progressive, moderate to severe | severe | no improvement, IS ongoing | 1 | 11 |
| Quiescent, any grade (even no cGvHD at this moment) | moderate to severe | no improvement, IS ongoing | 2 | 20 |
| De novo, moderate to severe | severe | no improvement, IS ongoing | 3 | 4 |
| Progressive, any grade (even no cGvHD at this moment) | any grade with or without improvement | improvement*, IS ongoing or tapered | 4 | 32 |
| Quiescent, any grade (even no cGvHD at this moment) | any grade with or without improvement | improvement, IS ongoing or tapered | 5 | 13 |
| De novo, any grade (even no cGvHD at this moment) | any grade with or without improvement | improvement, IS ongoing or tapered | 6 | 49 |
| | | | | |
| * or stable, if moderate cGvHD since the beginning | | | | |

| Multivariate analysis on OS | | |
|---|---|---|
| | HR (95% CI) | p |
| Group 0 | 1 | |
| Group 1 | 2.00 (1.00 - 4.01) | 0.05 |
| Groups 2&3 | | n.s. |
| Groups 4&5&6 | 0.23 (0.14 - 0.41) | <0.0001 |
| | | |
| Multivariate analysis on TRM | | |
| | HR (95% CI) | p |
| Group 0 | 1 | |
| Groups 1&2&3 | 2.38 (1.29 - 4.42) | 0.006 |
| Groups 4&5 | | n.s. |
| Group 6 | 0.30 (0.10 - 0.90) | 0.03 |
| | | |
| Multivariate analysis on relapse/progression | | |
| | HR (95% CI) | p |
| Group 0 | 1 | |
| Groups to 1 to 6 | 0.24 (0.11 - 0.53) | <0.0001 |
| | | |
| Variables tested: patient's age, HLA matching between patient and donor, stem cell source, disease status before HSCT, comorbidity score | | |
| Patient's age (cont.) was the only significant variable, in OS and TRM models | | |

Duration of IS was calculated as well as any change in cGvHD severity overtime, at 6-month intervals, with the exception of first 3 months after HSCT for those patients presenting cGvHD at this moment. cGvHD characteristics were divided into six groups according to presentation, severity and response to IS at 6 and 12 months after HSCT (Table 1). Correlation with TRM, OS and relapse/progression was evaluated by univariate and multivariate Cox model after adjustment for main transplant variables.
Results: On 313 transplanted patients transplanted in the above mentioned period,130 developed cGvHD and 129 were evaluable. Multivariate Hazard Ratios on OS, TRM and relapse/progression are shown in Table 2. A moderate or severe cGvHD persisting without improvement at 12 months after HSCT is predictive of significantly higher TRM and inferior OS.Importantly, forms of cGvHD that are responsive to IS and that allow complete or partial tapering of IS within 12 months after HSCT were associated with better outcome, despite a moderate or severe initial presentation (Table 2).
Conclusion: Not only severity but also presentation of cGvHD and duration of IS showed to be predictive of TRM and OS after HSCT; in particular, response to IS and cGvHD severity at 12 months post-HSCT seem to be predictive of final outcome. The present approach allows to better describe the complexity of post-transplant reality, where severity and duration of IS can change overtime and are associated with patients' outcome. Multi-state model will be further used and implemented to better assess risk changes according to post-transplant events.

**P1052**

***In-vivo* T-cell depletion with rabbit anti-thymocyte globulin prevents severe acute- and chronic-graft-versus host disease and allows safe allogeneic stem cell transplantation from mismatched, unrelated donors**

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Transplant options are limited for adult patients (pts) who lack a fully matched related or unrelated donor. We hypothesized that *in-vivo* T-cell depletion with thymoglobulin (thymo) would allow safe allogeneic stem cell transplant (allo-SCT) from mismatched, unrelated donors, thus expanding the potential donor pool for pts with hematologic malignancies who require allo-SCT.
Patients and Methods: Thirty eight adult pts (age 20–70, median 46) underwent a first unrelated, mismatched, allo-SCT between 1/1/2006 and 9/30/2011 at Mayo Clinic Arizona for hematologic malignancy (36 PBSC, 2 marrow). All pts had at least a one allele or one antigen mismatch (MM) at HLA-A, -B, -C, or -DRB1, and all except one pt received thymo as part of the GVHD prophylaxis strategy. One pt received Campath after experiencing anaphylaxis to thymo. Pts were transplanted for AML (n=20), ALL(8), CML (1), MDS (5), or NHL (4). Conditioning was myeloablative in 20, reduced intensity in 18. Mismatches were as follows: 1-allele MM (11); 1 antigen MM (19); 1 antigen, 1 allele MM (5); and 2 antigen MM (3). Additional GVHD prophylaxis included tacrolimus plus either methotrexate (n=22), mycophenolate mofetil (n=14), or other (n=2).
Results: The median follow-up for surviving pts is 16 months. As of 11/30/11, 30 pts were alive, and 8 had died (4 NRM, 4 relapse). There have been no deaths related to acute or chronic GVHD. The 1- and 2-year estimated rates of overall survival are 83%/77%; of progression-free survival 79%/71%. The estimated rate of relapse at 1 and 2 years is 12%/19%, and of non-relapse mortality 9.3%/12.4%. Four pts (10.8%) have developed severe (grades III-IV) acute GVHD. Moderate to severe NIH-defined chronic GVHD occurred in a single pt at risk. Five pts have reactivated EBV, with two developing PTLD (one late death possibly related to EBV-PTLD). CMV reactivation was seen in 24 pts (65%), CMV disease in 4, with no deaths directly related to CMV.
Conclusions: *In vivo* T-cell depletion with rabbit ATG (thymoglobulin) abrogates severe acute and (particularly) chronic GVHD, and allows use of mismatched, unrelated donors for allo-SCT in adult pts with otherwise incurable hematologic malignancies. Long-term survivors are generally free of severe chronic GVHD, with good quality of life. There does not appear to be an increased incidence of disease relapse, and non-relapse mortality is low. This approach is safe, effective, and considerably expands the donor pool for adult pts who require allo-SCT.

**P1053**

**Growth factor-associated graft-versus-host disease and mortality 10 years after allogeneic bone marrow transplantation**

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We analyzed the effects of growth factor on outcome after HSCT with >9 years of follow-up. Of 1,887 adult patients with acute leukaemia who received bone marrow from HLA-identical sibling donors and who were treated with myeloablative conditioning, 459 (24%) were treated with growth factor.
Growth factor hastened engraftment of neutrophils (p<0.0001), but reduced platelet counts (p=0.0002). GVHD-free survival (no acute GVHD of grades II–IV or chronic GVHD) at 10 years was 12±2% (±SE) in the growth factor group, as opposed to 17±2% in the controls (hazards ratio (HR) 0.81, p=0.001). Similar differences in GVHD-free survival were seen in patients with or without conditioning with total body irradiation (TBI).
Non-relapse mortality (NRM) was higher in the growth factor group irrespective of whether or not there was conditioning with TBI (HR=1.48; 95%CI: 1.15-1.9; p=0.002; HR=1.59; 95%CI: 1.07-2.37; p=0.02, respectively). Both groups had similar probabilities of leukaemic relapse (HR=0.96; 95%CI: 0.78-1.18; p=0.71).
Leukaemia-free survival (LFS) at ten years was 35±2% in those receiving growth factor prophylaxis, as opposed to 44±1% in the controls (HR=0.70; 95% CI: 0.60-0.82; p=0.00001).
Prophylaxis with growth factor increases the risk of GVHD, does not affect relapse, increases NRM and reduces LFS more than 10 years after HSCT, regardless of conditioning with TBI.

**P1054**

**B-cell activating factor and reconstitution of B-cell compartment after allogeneic stem cell transplantation: impact on chronic graft-versus-host disease developement**

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Objectives of the study: On the basis of previous evidences of B cell compartment involvement in patients with chronic Graft versus Host Disease (cGvHD), we investigated recovery and chimerism of B lymphocytes and circulating levels of B cell activating factor (BAFF) cytokine after allogeneic hematopoietic stem cell transplantation (HSCT).
Methods: We prospectively analyzed 82 hematological malignancy patients, median age 51 years (range 20-69), who underwent HSCT. We evaluated immunological recovery by flow-cytometry, hematopoietic chimerism by STR-PCR in whole peripheral blood (PB), granulocyte fraction and immunomagnetically sorted CD19+ cells, and BAFF serum levels before HSCT and at day 90, 120 and 180.
Results: Forty-three patients developed cGvHD at a median of 5 months after HSCT, with at least 2 organs involved and a median score of 3 according to the Organ Scoring System. In 25 cases the cGvHD progressed from a pre-existent acute GvHD.

We could not analyzed B cell chimerism at day 90 in more of 80% of cases, due to the low count of CD19+ cells, but at day 120 we found full donor chimerism (FDC) on CD19+ cell fraction of all patients who had reached FDC in the whole PB and in the granulocyte fraction.
The CD19+ cell counts were similar between the two groups through the first 120 days, but they increased at 180 day more in cGvHD negative patients then in positive ones (141/µl versus 61/µl).
BAFF kinetics was similar in the two cohorts. BAFF levels raised to a peak at day 90 in both groups (7368 pg/ml in cGvHD- versus 7764 pg/ml in cGvHD+) and then progressively decreased at day 180 (respectively 6157 pg/ml and 6422 pg/ml). Mean BAFF/CD19+ cell ratio was lower in the cGvHD negative patients at 180 days (406 versus 119) and this correlates with a faster B cell recovery.
Conclusions: Although severe B lymphocytopenia persisted through the first 180 days in patients with and without cGvHD, the B cell compartment presented a complete donor engraftment ad day 120 in all patients. We observed that patients who do not developed cGVHD achieved a more rapid B cell recovery and showed a lower BAFF/CD19+ ratio. In contrast, B cell recovery was delayed and high BAFF/CD19+ B cell ratios persisted in patients who developed cGVHD.

**P1055**

## HSPA1L and HSPA1B mRNA expression in the pathogenesis of graft-versus-host disease

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Background: An adverse outcome of allogeneic haematopoietic stem cell transplantation (allo-HSCT) is Graft versus-Host Disease (GvHD). Heat shock proteins have been shown to exhibit an important role under stress conditions. HSP70 (HSPA) family comprises of three members; HSPA1A, HSPA1B and HSPA1L and has been shown to be involved in inflammation and allograft rejection. HSPA1A/HSPA1B (HSP0i) are inducible while HSPA1L is constitutively present. In the *in vitro* skin explant assay (SEA) the association of HSPA1B and Graft-versus-Host Reactivity (GvHR) has been established. The aim of this study was to investigate whether HSPA1B and HSPA1L messenger RNA (mRNA) expression levels in whole blood and HSP0i antigen and antibody levels in sera correlate with GvHD severity.
Methods: Whole bloods (n=71) were collected in PAXgene tubes 7 days before transplant and at set time-points after transplant (Day 28 to 12 months) and used for total RNA

**[P1055]**

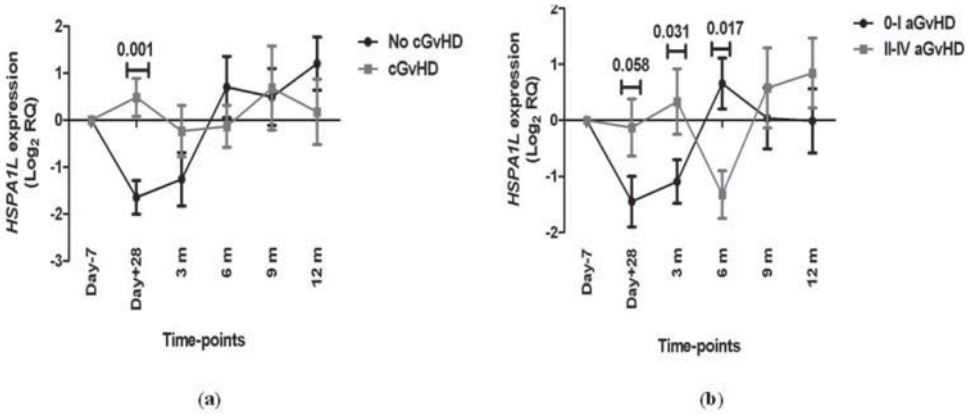


**Figure 1. (a)** *HSPA1L* mRNA expression in whole blood of no chronic GvHD versus chronic GvHD patients. **(b)** *HSPA1L* mRNA expression in whole blood of 0-I aGvHD versus II-IV aGvHD patients. Pre-transplant time-point (Day -7) was considered as the calibrator

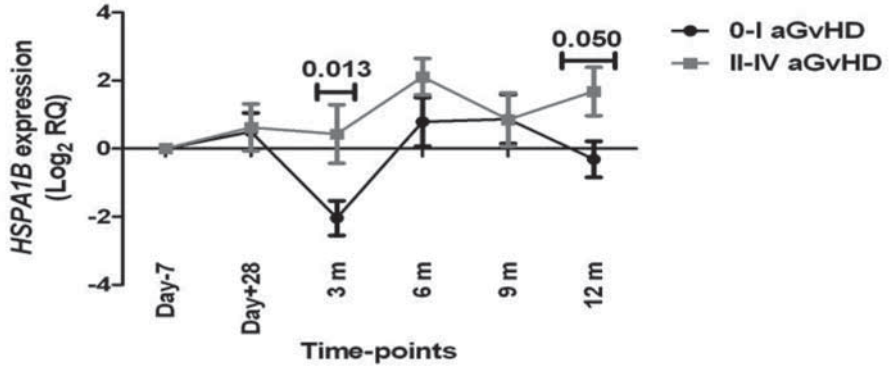


**Figure 2.** *HSPA1B* mRNA expression in whole blood of 0-I aGvHD versus II-IV aGvHD patients.

extraction. HSPA1B and HSPA1L mRNA levels were measured using Taqman gene expression assays. GAPDH was used as the endogenous control for mRNA studies. Enzyme-linked immunosorbent assay (ELISA) was used to detect HSP0i antigen (n=16) and HSP0i antibody (n=52) concentrations in sera of allo-HSCT patients at various set time-points; pre- and post-transplant (Day -7 to 12 months).
Results (Whole Blood-Gene Expression): HSPA1L mRNA expression was up-regulated at 28 days post-transplant (p=0.001) in chronic GvHD (cGvHD) patients (Figure 1a). In addition, in the II-IV acute GvHD (aGvHD) patients there was significantly higher HSPA1L mRNA expression at 3 months post-transplant (p=0.031), however a down-regulation was observed at 6 months post-transplant (p=0.017) (Figure 1b). No significant variation in HSPA1B was observed for cGvHD patients but its expression was significantly higher at 3 months post-transplant (p=0.013) in the II-IV aGvHD group (Figure 2).
Results (Sera-ELISA): No significant variation was observed between HSP0i antigen levels of either the aGvHD (0-I versus II-IV) or cGvHD patients. However, in 0-I aGvHD versus II-IV aGvHD patients there was a trend for lower anti-HSP0i levels at 3 months post-transplant (p=0.060). Lower levels of anti-HSP0i were also observed at 6 months post-transplant (p=0.0158) in the cGvHD patients.
Conclusion: Our study has shown that HSPA1L can be an early predictor of cGvHD while due to increasing mRNA expression post transplant; HSPA1B may be involved in aGvHD development, and disease progression.

**P1056**

**Biopsy-verified bronchiolitis obliterans predicts irreversibly reduced lung function as ppposed to other non-infectious inflammatory pathology**

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Bronchiolitis Obliterans (BO) affects 2-26% of patients after allogeneic stem cell transplantation (SCT). Diagnostics can be difficult due to the insidious course. Lack of consistent clinical criteria has lead to great variability in reported outcome. Although lung biopsy remains the diagnostic gold standard,

**[P1056]**

Table 1: Summary of pulmonary function tests in bronchiolitis obliterans (BO) patients, matched controls and patients with other non-infectious pulmonary pathology (NIPP).

| | | Baseline % (n) | 3-12 months % (n) | 1-2 year % (n) | 2-3 years % (n) | 3-5 years % (n) |
|---|---|---|---|---|---|---|
| FEV1% predicted | BO | 92.7 (22) | 76.2 (21) (p=0.006)* | 64.8 (19) (p=0.002)* | 63.4 (18) (p=0.004)* | 64.1 (15) (p=0.012)* |
| | Controls | 96.8 (41) | 94.8 (37) | 91.6 (24) | 94,0 (22) | 97.3 (22) |
| | NIPP | 98.9 (26) | 83.1 (24) (p=0.003)* | 74.7 (18) (p=0.003)* | 83.4 (14) | 85.5 (12) |
| FEV1/FVC ratio | BO | 83.6 (22) | 76.2 (21) (p=0.005)* | 67.6 (19) (p=0.002)* | 65.4 (18) (p=0.001)* | 63.5 (15) (p=0.003)* |
| | Controls | 82.4 (41) | 82.6 (37) | 80.7 (24) | 81.8 (22) | 79.5 (22) (p=0.012)* |
| | NIPP | 80.9 (26) | 79.9 (24) | 79.2 (18) | 78.9 (14) | 75.1 (12) |
| MMEF75-25 % predicted | BO | 75.9 (15) | 59.4 (14) (p=0.03)* | 43.0 (15) (p=0.028)* | 39.7 (17) (p=0.05)* | 39.6 (14) |
| | Controls | 80.1 (27) | 80.1 (25) | 82.2 (18) | 76.8 (14) | 82.5 (16) |
| | NIPP | 98.7 (15) | 89.1 (15) | 68.4 (10) (p=0.015)* | 63.4 (10) | 71.3 (12) |
| DLCO % predicted, Hb corrected | BO | 72.1 (16) | 57.6 (21) (p=0.006)* | 58.7 (17) (p=0.006)* | 57.7 (15) (p=0.016)* | 63.2 (14) |
| | Controls | 77.5 (27) | 69.5 (32) (p=0.009)* | 68.5 (18) (p=0.016)* | 75 (16) | 78.6 (20) |
| | NIPP | 76.2 (24) | 53.8 (24) (p<0.001)* | 51.6 (18) (p<0.001)* | 53.5 (13) (p=0.005)* | 58.5 (12) (p=0.05)* |

* Compared to baseline values (Wilcoxon signed rank sum test).

FEV1 forced expiratory volume in 1 second, FVC forced vital capacity, MMEF75/25 maximum mid-expiratory flow between 25 and 75% of FVC, DLCO diffusion capacity for carbon monoxide, Hb haemoglobin

biopsy-based studies are scant. This study describes the course of BO in a national cohort of pathologically confirmed cases.
Methods: A case-control study in 798 patients from the Danish National SCT-cohort 2000 to 2010 was performed. BO was diagnosed in 23 cases (3%) age 1-62 years by a single experienced pathologist. Cases were compared to 46 controls matched by age, donor type, stem cell source, conditioning regime, diagnosis and year of SCT. Twenty-nine patients (4%) were diagnosed with non-infectious pulmonary pathology (NIPP) other than BO. Median time from SCT to biopsy was 340 days (range 99-1356) and 287 (84-2557), respectively.
Results: In BO patients FEV1, FVC, FEV1/FVC, MMEF75/25 and DLCO declined from baseline until 1-2 years post-SCT. At this point the lung function stabilized at a reduced level (Table 1).
Compared with matched controls lung function was reduced in BO patients 3-12 months post-SCT. After 3-5 years there was still a difference in FEV1 (p=0.002), FVC (p=0.009), FEV1/FVC (p=0.003), MMEF75/25 (p=0.001) and DLCO (p=0.025). The frequency of cGvHD in other organs was higher within the BO group than in controls at 2-3 years post SCT (56.3 *vs.* 22.2%, p=0.025).
Compared with the NIPP group BO patients had a lower FEV1 (52.5 *vs.* 66.2% p=0.031), FEV1/FVC (67.6 *vs.* 78.1%, p=0.04) and MMEF75/25 (34.3 *vs.* 72.6% p=0.015) at time of biopsy. NIPP-patients experienced an initial decline in FEV1, FEV1/FVC, MMEF75/25, and DLCO, which was subsequently ameliorated leaving no significant difference from baseline after 3-5 years. BO patients had lower FEV1 than NIPPs 3-5 years post SCT (p=0.047). No difference was seen in cGvHD in other organs or level of immunosuppression. Survival did not differ between the groups compared (p=0.7).
Conclusion: Biopsy-verified BO is a predictor of irreversibly reduced lung function following SCT. Patients with other non-infectious pathology have an initial fall in lung function, but tend to improve and stabilize at pre-SCT levels. Whether earlier diagnoses and thus earlier initiated treatment might halt the decline and lead to better preservation of lung function in BO-patients is topic for further research.

**P1057**
**Failure of improvement in BOS in ECP treated cGvHD – a single-centre experience**
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Bronchiolitis Obliterans Syndrome (BOS) is characterized by new development of fixed airflow obstruction after allogeneic haemopoietic stem cell transplant (HSCT) with a prevalence of 5.5% post HSCT and 14% in patients with chronic graft-versus-host disease (cGVHD). The prognosis of refractory BOS remains poor with an overall survival of 13% at 5 years. Given the limited success with traditional treatment, Extracorporeal Photopheresis (ECP) has been used as a therapeutic option, with a suggestion that it has a stabilizing effect on BOS.
Aim: To analyse the impact of ECP on patients identified with BOS in terms of improvement in Pulmonary Function Tests (PFTs) and clinical symptoms with a retrospective case note review.
Methods: In our institution we reviewed the records of 186 patients referred over a 14 year period and applied the NIH criteria to identify patients with BOS. ECP was delivered as a paired treatment every fortnight for a 14 week assessment period, which based on response, was reduced to 4 weekly.
Results: We identified 9 patients (6 male, 3 female) who at the time of initiation of ECP met the diagnostic criteria for BOS. The median age was 42 years (range 17-51 yrs). The underlying diagnoses were AML=5, ALL=1, Primary Myelofibrosis=1, CML=1 and NHL=1. In 5 TBI was part of the conditioning regime. 3 underwent matched unrelated donor transplant and 1 underwent haploidentical transplant. All patients had additional non-respiratory cGVHD with 3 having >3 sites involved. The median time to initiation of ECP was 27 months (range 12-60 months) with a median follow up of 170 months (11-479). The median number of immunosuppressants used was 3 (range 2-5). Only 1 patient (11%) showed an objective improvement based on PFTs. One patient was able to be weaned off supplemental oxygen despite not showing an improvement in PFTs. No significant improvements in PFTs or symptoms were noted in the remainder of the group.
Conclusion: Though ECP is effective in certain forms of steroid refractory cGvHD, our data suggest that its role in the treatment of BOS remains limited and needs further evaluation. We were unable to demonstrate a stabilization of PFTs as previously reported (Lucid, 2011). More effort needs to be focused on early detection of respiratory GVHD with a stringent PFT screening strategy post HSCT. Biomarkers may also have a role to play in help identifying those patients who may be poor responders to ECP and may require alternative therapy (Whittle, 2011)

**P1058**
**A promising therapy for Bronchiolitis obliterans syndrome after lung transplantation is the extracorporeal photopheresis**
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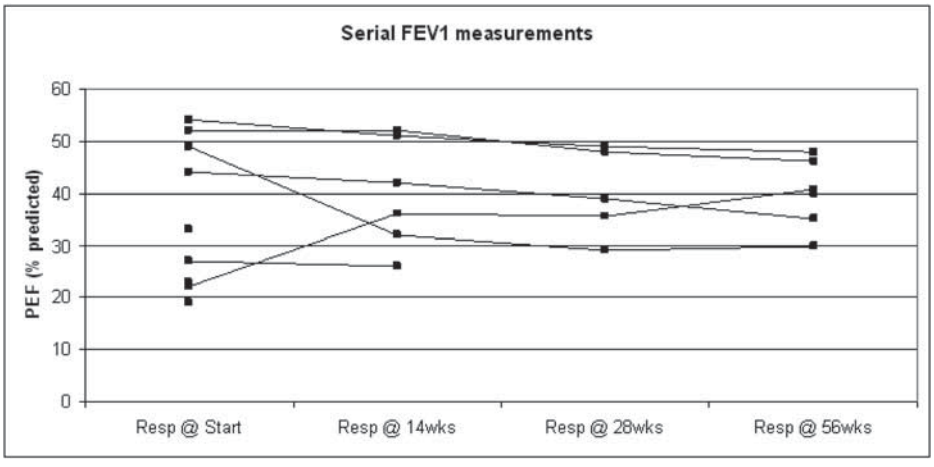
Introduction: Bronchiolitis obliterans syndrome (BOS), the manifestation of chronic allograft rejection in patients after allogeneic lung transplantation for end-stage lung disease, is a severe and life threatening complication with an unfavourable

**[P1057]**



long-term outcome. Within 5 years of BOS onset most patients die of respiratory failure due to progressive decline in pulmonary function. Disease progression might be slowed down by modern immunosuppressive regimens however include the side effects of increased immunosuppression. An innovative therapeutical option for BOS is the Extracorporeal photopheresis (ECP) and data about the efficacy of ECP in BOS are limited.
Methods: A 33 year-old male patient with severe hereditary pulmonary fibrosis presented with a BOS 42 months after double-lung transplantation. Even under a triple drug therapy with steroids, a calcineurin inhibitor and a cell-cycle inhibitor and additional steroid boli he experienced a progressive decline in lung function. With a BOS grade 3 basing on a vital capacity (VC) of 5,100 ml (former BOS grade 1: VC of 5,940 ml) and a forced exspiratory volume in one second (FEV1) of 2,120 ml (former BOS grade 1: FEV1 of 4,930 ml) he was transferred to our unit for photopheresis therapy. A Vortex™ port was implanted and thereafter an intensive ECP treatment twice weekly for 8 weeks was started. Then the ECP treatment schedule was changed to twice weekly every second week for 10 weeks, thereafter twice weekly every third week for 9 weeks and then extended to twice weekly every month.
Results: The patient tolerated well the intensive ECP therapy with no severe infectious disease complications during the ECP treatments. Twelve weeks after begin of the ECP no further decline of lung function was measured by spirometry with a VC of 5,070 ml and a FEV1 of 2,160 ml. Furthermore, 27 weeks after begin of the ECP treatment an improvement of the lung function with a VC of 5,360 ml and a FEV1 of 2,410 ml was confirmed.
Conclusion: Even the intensive ECP treatment is well tolerated by the patient. Furthermore the immunosuppressive medicinal therapy going along with toxicity and an increased risk of infectious diseases can be reduced under ECP treatment. Beyond that intensive ECP treatment might slow down the rate of decline in lung function and moreover might even improve lung function. Therefore, an innovative promising therapeutic option in patients with BOS after lung transplantation is the ECP therapy.

**P1059**

**Extracorporeal photopheresis for pulmonary graft-versus-host disease – results from an observational audit**

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Background: Chronic graft-versus-host disease (cGvHD) of the lung is a devastating late-onset non-infectious pulmonary complication of allogeneic hematopoietic stem cell transplantation (HSCT), with high morbidity and mortality. Treatment options are limited to immunosuppression, antiinflammatory and anti-obstructive drugs, inhibitors of the platelet-derived growth factor receptor (PDGFR)/transforming growth factor beta (TGFb) pathways, or lung transplantation. Here, we present results from an observational audit on the use of extracorporeal phototherapy (ECP), a modality with promising activity in other cGvHD manifestations, in the setting of pulmonary cGvHD.
Patients and Methods: Twenty-seven patients received ECP for pulmonary cGvHD after allogeneic HSCT for AML (15), CML/CMPN (6), ALL (3) or lymphoma (3). Median age at transplant had been 40 years (range: 16 to 59); 10 patients were female and 17 male. Initial severity of pulmonary cGvHD according to NIH criteria was mild (3), moderate (17), or severe (7 patients). All patients had already been treated with 2 to 8 (median 4) lines of therapies including steroids, calcineurin inhibitors, mycophenolate, sirolimus, imatinib, macrolides, beta-2-mimetics, or montelukast, leading to improvement (6), no change (16), or deterioration (5 patients). ECP was started with the intent to (i) either stabilize rapidly declining lung function in patients with severe pulmonary cGvHD, or (ii) improve patients with stable but unsatisfactory prior results. Response to ECP was evaluated by lung function testing, according to NIH criteria.
Results: Over a period of 2 to 76 (median 15) months, 5 to 80 (median 23) ECP cycles were applied to every patient. Decline of lung function was stopped in all 5 patients with prior progressive severe pulmonary cGvHD. 4 of 6 patients with stable severe pulmonary cGvHD improved to moderate severity. Conversely, only 2 patients with stable mild and none of 9 patients with stable moderate pulmonary cGvHD showed any significant further improvement after ECP. 21 of 27 patients are still alive after a median follow-up of 46 (range: 6 to 143) months from diagnosis of pulmonary cGvHD; overall survival is 80% after 5 years and 64% after 10 years, which compares favourably with historical controls.
Conclusion: ECP appears to represent a valuable therapeutic rescue option, especially for patients with severe pulmonary cGvHD. Prospective studies are warranted to confirm these encouraging findings.

**P1060**

**Impact of extracorporeal photopheresis for treatment of chronic graft-versus-host disease on iron overload**

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Background: Recently, extracorporeal photopheresis (ECP) has been proposed as an efficacious and safe alternative therapy for patients with steroid refractory/dependant chronic Graft versus Host Disease (cGVHD). No clinically significant ECP-related side effects are reported. Iron overload, primarily related to red blood cells transfusions, is relatively common complication in hematopoietic cell transplant survivors.
Aims: To asses iron balance in ECP serially treated transplanted patients.
Methods: From January 2000 to November 2011, 40 patients (M/F: 23/17) affected by steroid refractory/dependent cGvHD and treated with ECP are evaluated. Median age at transplant was 41 years (range: 4-62). Thirty-seven (92.5%) and 3 (7,5%) patients were transplanted for malignant and non-malignant haematological disease, respectively. The median interval from cGvHD diagnosis to ECP was 7 months (range 0-62); all patients received at least two prior lines of immunosuppression including cyclosporine, steroid, micophenolate-mofetil and tacrolimus. Patients were treated on 2 consecutive days (one cycle) at 1 week interval for the first month, at 2 weeks interval for the second month and at 4 weeks interval subsequently. Baseline and bimonthly evaluation of iron metabolism (plasma iron, ferritin, transferrin) was carried out.
Results: A median number of 20 cycles (range: 7-39) was performed with a median treatment duration of 14 months (range: 3-153). Median baseline and post-ECP ferritin was 822 ng/ml (range: 160-12685) and 180 ng/ml (range: 9-1851), respectively (p=0.01). Eleven (22%) patients developed an iron deficiency; median baseline and post-ECP ferritin were 95 ng/ml (range: 39-1228) and 11 ng/ml (range: 9-20), respectively. Furthermore, iron deficiency was confirmed by both median plasma iron [32 mcg/dl (range: 14-50)] and transferrin [401 mg/dl (range: 287-449)]. All patients with iron deficiency received iron supplementation to prevent the development of anemia. Occult blood losses or inadequate iron intake were excluded in this series.
Conclusions: Iron overload is a common transplant related complication. No data are available to asses the natural evolution of post-transplant iron overload. In our experience prolonged ECP therapy is associated to significant decreases in serum ferritin. A careful assessment of iron metabolism during ECP is therefore necessary to prevent the onset of iron deficiency in the patients with basal ferritin not suggestive of iron overload.

**P1061**

**Efficacy and infectious complications during extracorporeal photopheresis treatment of chronic graft-versus-host disease**

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Objective: cGVHD occurs in approximately 20 to 70% of patients after allogenic hematopoietic cell transplantation (allo-HCT) and requires long-term systemic immunosuppressive treatment. ECP is effective method of resistant form cGVHD, but infectious complications (IC) and related toxicity are present.
Methods: The study included 30 patients (pts) with extensive cGVHD after allo-HCT. The age was from 2 to 55 y.o. (median 21 y.o.). ECP was used as second or third line therapy. 14 pts have unrelated donors, 16 pts have related donors. HLA-matched: matched donors in 20 cases (66%), mismatched in 3 (10%) and haploidentical in 7 (24%). According to NIH criteria 24 (80%) pts had severe cGVHD and 6 (20%) pts had moderate cGVHD. De novo cGVHD was in 3 (10%), progressive in 7 (24%), overlap syndrome in 3 (10%) and quiescent cGVHD in 17 pts (56%). Eligibility criteria for ECP were steroid-dependence in 15 (50%) pts, steroid-resistance in 9 (30%) pts and steroid-intolerance in 6 (20%) pts.
Results: Complete response (CR) was observed in 3 pts (10%), partial response (PR) in 18 pts (60%) and absence of response was in 9 pts (30%). IC as de novo was identified in 22 pts (73%) of them: bacterial – 14 (46%), reactivation of CMV-infection – 7 (24%), invasive mycosis – 21 pts (70%).
Probability of overall survival (OS) at 3 years was 83%. In patients with IC probability of OS was 77%, in pts without – 80%. In patients with CR of steroid-refractory cGVHD probability of OS at 3-years was 100%, in patients with PR OS at 3-years was 93% and in patients without response to ECP OS at 3-years was 52% (p-0,107).
Conclusion: ECP is an effective therapy resulting in significant response rate in steroid-refractory cGVHD. In spite of ECP has infectious complications that don't influence on OS. Overall probability of survival is more affected to treatment response – complete or partially. This method should be used as a part of combined immunosuppressive therapy of cGVHD.

**P1062**

**Comparisons of the cellular subsets obtained using the CELLEX™ and XTS™ devices during extracorporeal photopheresis therapy in adults and children with chronic graft-versus-host disease**

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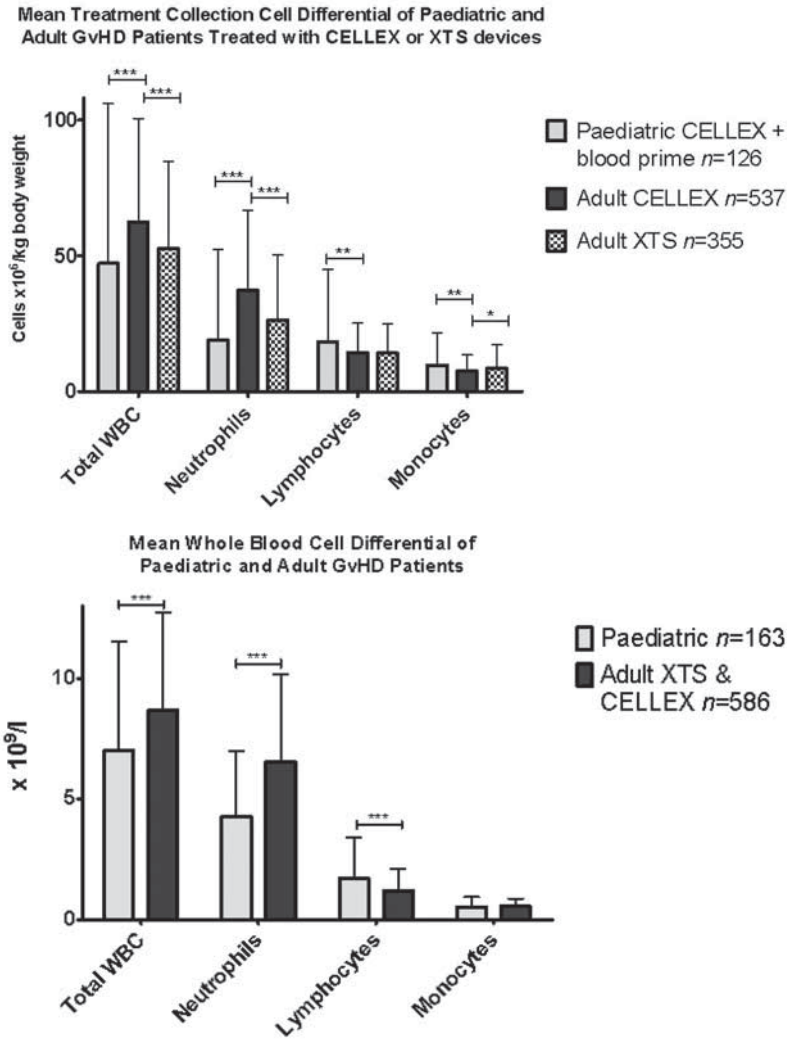
Objectives: Extracorporeal photopheresis (ECP) is used as a safe and efficacious treatment for steroid-refractory chronic graft versus host disease (cGvHD). ECP involves the separation

**[P1062]**



of white blood cell (WBC)-rich plasma, followed by *ex-vivo* administration of a photosensitiser and ultraviolet-A radiation, before reinfusion. Two closed THERAKOS™ Photopheresis Systems are used: the UVAR™ XTS™; and more recently, the CELLEX™. The mechanism of ECP is not fully elucidated, and the potential influence of the treatment bag cell differential is of interest. We examined the relationship between peripheral blood WBCs and the cells harvested in the bag, compared adult and paediatric collection compositions, and whether the XTS™ and CELLEX™ devices were similar in their collection of cellular subsets.
Methods: Samples were taken from: 537 treatment collections from 70 adults with GvHD undergoing ECP therapy using the CELLEX™ device; 355 collections from 45 adults treated with the XTS™; and 126 collections from 16 children treated with the CELLEX™ incorporating a blood prime. Total WBC counts and cell differentials (neutrophils, lymphocytes and monocytes) were obtained.
Results: In the peripheral blood, adult patients had significantly higher mean numbers per kilogram body weight (kg bw) of total WBCs and neutophils, than children, and lower lymphocytes. This was consistent with the differences found between collection samples from CELLEX™-treated adults and children, plus mean monocytes per kg bw were greater in children. Comparisons of the 2 devices revealed higher mean total WBC and neutrophil, and lower monocyte numbers per kg bw, in adult collections obtained with CELLEX™ use, compared to the XTS™. No differences were found in adult collection mean lymphocyte numbers per kg bw between the 2 devices.
Conclusion: WBCs collected during ECP therapy are representative of the peripheral blood population, which we found differed between paediatric and adult patients. Not all differences between adult and paediatric collections were attributable to the peripheral blood composition, however, as collections from children were higher in mean monocytes per kg bw. Despite the smaller volume of blood we treat during ECP in children, the mean numbers of lymphocytes and monocytes obtained per kg bw were similar to adult values. The XTS™ and CELLEX™ devices yielded similar mean lymphocyte numbers per kg bw in adults.

**P1063**

**Mature circulating endothelial cells and progenitors in patients with chronic GvHD**

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Acute and chronic graft-versus-host disease are a common complication of allogeneic stem cell transplantation (ASCT). In animal models acute GVHD (aGVHD) is associated with increased neovascularization and number of circulating endothelial cells (CECs), while patients with sclerodermatous chronic GVHD (cGVHD) show a significant decrease in the number of circulating endothelial progenitor cells (EPCs) in peripheral blood as compared to patients with non sclerodermatous cGVHD or controls.
To evaluate the role of CECs and EPCs in patients with cGVHD, we analysed a total of 15 patients affected by hematological malignancies having undergone ASCT following reduced intensity conditioning. Donors were HLA identical in 14 patients and HLA aploidentical in 1. Acute GVHD and cGVHD were defined on the basis of time of manifestation, ≤100 days for aGVHD and >100 days for cGVHD. At the time of the blood sample collection, 8 patients, median age 42 years (28-51), with a median time after transplant of 177 days (21-1373), had no evidence of GVHD; of those 5/8 were evaluable for aGVHD and cGVHD, 2 only for aGVHD; 4/8 were on calcineurin inhibitors immunosuppressive therapy; 7 other patients, median age 51 years (38-64), with a median time after transplant of 844 days (314-1779), were all evaluable for acute and cGVHD and had evidence of cGVHD as follows: sclerodermatous in 3 patients requiring systemic immunosuppressive therapy, oral mucosa lichen in 1 patient taking oral corticosteroid and cutaneous erythematous and dischromic cGVHD in the other 3 patients, with only 1 patient on systemic immunosuppressive therapy. Viable and apoptotic CECs and EPCs were evaluated by six color flow cytometry. Briefly, CECs were defined as DNA+CD45-CD31+ CD146+, EPCs as CD45- CD34+. The combination of Syto16 and 7-AAD was used to discriminate between viable (syto16bright/7-AAD-) and apoptotic (syto16weakly pos/7-AAD+) endothelial cells, and to exclude from analysis, platelets and endothelial macroparticles.
The results, expressed as median of cells/mL, are summarized in Table 1.
These preliminary data indicate a significant reduction in apoptotic circulating mature endothelial cells, likely reflecting a poor vascularization of multiple organs and tissues targeted by cGVHD and a trend towards a decreased number of EPCs in patients with cGVHD. A multicentric study is now planned to

**[P1063]**

Table 1.

| | Total CECs | Viable CECs | Apoptotic CECs | EPCs |
|---|---|---|---|---|
| Healthy Subject | 103 (33-322) | 21 (3-67) | 77* (28-303) | 31 (0-56) |
| Patients with cGVHD | 46 (29-94) | 41 (25-68) | 5* (3-26) | 30 (0-213) |
| Patients without GVHD | 138 (30-179) | 39 (10-153) | 68* (5-136) | 49 (0-355) |
| | | | *p<0.017 | |

confirm these hypotheses and investigate a possible predictive/ prognostic role of CEC and EPC in cGVHD.

**P1064**

**Role of circulating TH-17 lymphocytes in patients undergoing allogeneic stem cell transplantation**

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Introduction: It has been recently described a subpopulation of CD4 + T cell, called Th17, producing IL-17 and IL-21 and IL-22. Circulating Th17 are increased in inflammatory processes but their role in the development of GVHD, is not elucidated. In this work we quantified circulating Th-17 in patients undergoing allo-HSCT.
Patients and Methods: In this transversal study, we included 41 patients. Median age was 46 years, 48.8% received reduced-intensity conditioning (RIC) and 56.1% were grafted with peripheral blood stem cells. Out of them, 16 patients had active chronic GHVD, 1 patient acute GVHD and 24 patients had no signs/symptoms of GVHD. We included as controls 7 healthy donors. MNCs were stimulated with PMA and ionomycin and inhibition of degranulation we carried out using Brefeldin A. Th-17 were quantified by flow cytometry using the following MnAbs: Anti-CD4, Anti-IL17A, Anti-IL-17F, Anti-IL-22 and Anti-IL-21 (all from eBioscience) and for Th1 responses Anti-IFN (from BD). At least $1x10^4$ CD4 + lymphocytes were acquired.
Results: Mean percentage±SE of Th-17 over total CD4+ lymphocytes (IL17+INF-) was 1.14±0.35% in samples obtained from patients with GVHD, 0.56±0.09% in those without GVHD whereas it was 0.01±0.001% in healthy controls (P=.042). Mean percentage±SE of Th1 detection (IL17-IFN+) is 20.06±3.92% in patients with GVHD, 10.181±2.49% in patients without GVHD and 3.62±0.67% in healthy controls (P=0.004). On the other hand, patients who developed chronic gastrointestinal GVHD had more %Th-17 cells than cGVHD at other locations (P=.001). Patients who were treated with cyclosporine had more Th-17 cells (IL17+INF+) than those treated with other drugs (p=0.06). No IL-22 or IL-21 producing CD4+ lymphocytes were detected. When comparing Th17 values between different groups, we did not find significant differences with underlying disease, sex and donor age, degree of HLA disparity, treatment with ATG and GVHD prophylaxis. By contrast, the presence of Th-17 cells producing IFN- gamma (IL17+INF+) were higher with unrelated donors (p=.047), PB stem cells (p=.05) and the use of RIC (p=.09).
Conclusion: Patients undergoing allo-HSCT who develop chronic GVHD have a significantly increased proportion of Th-17 proinflammatory and INF-producing circulating lymphocytes. Therapeutic measures aimed to decrease or eliminate these cells could improve results in the treatment of chronic GVHD.
Funding Source: Ministry of Science and Innovation. BFU-2009-11286.

**P1065**

**CCR6 and CD138 co-expression on IL-17+ cells is different in cutaneous and mucosa membrane infiltrations in chronic GvHD**

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Objectives: Chronic graft-versus-host-disease (cGvHD) especially in the extensive form impairs the quality of life and makes people disable to continue normal activity due to inflammatory lesions in the skin and mucous. In this study we focused on the presence of IL-17+ cells and their CCR6 and CD138 positivity in affected tissues in association with clinical manifestations and serum CRP levels.
Methods: Twenty three biopsies were taken from 12 pts with cGvHD lesions after PBPC from sibling (n=7) or matched unrelated donors (n=5). Conditioning regimen (myeloablative - 5 pts or reduced intensity conditioning - 7 pts) included ATG - 9 pts and Campath - 1 patient. Skin was involved in all cases (poikiloderma and/or scleroderma-like lesions). Ten out of 12 pts had oral mucosa lesions and resulted in impairment of feeding and painful ulcerations (5/10 pts). Gastric and intestinal mucosa lesions resulted in vomits and diarrhea in 3 cases. Biopsy specimens: skin (n=14), gastrointestinal tract (n=6) and oral mucosa (n=3) were analyzed for the composition of cellular infiltrates (CD3, CD4, CD8, CD138) including detection of IL-17, FOXP3 and CCR6 positive cells. Co-expression of CCR6+/IL-17+ and IL-17+/CD138+ were verified by double immunostaining.
Results: (i) CD8+ prevailed over CD4+ lymphocytes in all oral and gut mucous biopsies but only in 8/14 skin biopsies (p=0.048). (ii) IL-17+ cells were seen in both mucosa (9/9) and skin biopsies (13/14) but in the mucosa they were significantly more frequent (p=0.007) with a median value >10 cells in 5 HPF. (iii) IL-17+ cells infiltrating the mucosa tissue were less frequently CCR6+ but more frequently CD138+ especially in the gut lesions as compared to the skin. (iv) FoxP3+ cells were rarely present at the tissue site but if seen their abundant presence was associated with mild clinical activity. (v) Presence of IL-17+ cells was associated with the severity of clinical manifestation and cases with tissue infiltrations rich in IL-17+ had more frequently elevated levels of serum CRP than those with scarce presence of IL-17+ cells (8/11vs 2/11 respectively; p=0.03).
Conclusions: CCR6 positivity of skin infiltrating IL-17+ cells and CD138 positivity of those in the gut suggest different mechanism of IL17+ cells accumulation. Activity of IL-17+ cells is associated with the inflammatory process reflected by elevated levels of serum CRP.

**P1066**

**Rituximab and total nodal irradiation in the treatment of extensive chronic graft-versus-host disease: interim results of a phase 2 study**

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Objectives: Chronic graft-vs-host disease (cGVHD) is the major cause of non-relapse mortality in patients (pts) surviving >100 days(d) after allograft. Despite this, there is no standardised salvage following first-line therapy, and novel approaches are

required. We prospectively evaluated the tolerability of combining rituximab and total nodal irradiation (R-TNI) in pts with extensive cGvHD after suboptimal responses to conventional systemic immunosuppression (SI).
Study design and patient characteristics: The primary endpoint was toxicity at 3 months(m) with response rate and durability of response as secondary endpoints. Pts with active or recurrent cGVHD despite maximally tolerable SI received a single dose of 1000 mg R followed by 1Gy TNI within 7d. Maintenance R (500 mg) at 3-monthly interval for 12m was provided for pts achieving a stable (1-49% reduction of SI) or partial (>50% reduction of SI) response at 3m. The median age of 19 study pts (treated from 2007-2011) was 48 years (range: 19-71); 13 had prior total body irradiation; 11 were matched unrelated donor transplants. All pts (except one) were receiving prednisolone at study entry (median dose of 25 mg/d) with 14 pts receiving concurrent two or more SI. The median time from allograft to R-TNI was 1124d (range: 187-2721). 17 pts had extensive skin cGvHD including 12 with predominantly sclerodermatous changes; two pts had refractory oral cGvHD; five pts had multi-organ disease. Seven pts were B-cell lymphopenic.
Results: Mild transient thrombocytopenia occurred in all pts (grade 1-2 n=18; grade 3 n=1). Neutropenia occurred in 11 pts (58%): grade 1-2 (n=8); grade 3-4 (n=3). Grade 3-5 sepsis was experienced by six pts (31%) including one death from bacterial septicaemia in week 9. Seventeen pts were evaluable for initial response at 3m: 6 pts (35%) had progressive cGvHD, while 11 pts (65%) had either stable (n=9) or partial response (n=2) with a median reduction of total SI by 25%. With a median follow up of 9m (range: 1-36), the median duration of response was 16m (range: 3-36) with one complete responder. Overall survival was 84% with three deaths (relapsed leukaemia; sepsis; cGvHD). During maintenance R, four pts with hypogammaglobinaemia and infection required initiation of IVIg, while one pt had transient grade 3 neutropenia.
Conclusion: For patients with extensive cGvHD inadequately controlled by SI, R-TNI induces transient tolerable haematological toxicity and achieves a modest rate of durable responses.

**P1067**
**A case of successful treatment of chronic GvHD with kidney involvement with rituximab**
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Membranous glomerulonephritis is a rare complication of hematopoietic cell transplantation (HCT). Here, we report the case of a 42-year-old woman with the diagnosis of AML with no chromosome abnormalities who received an allogeneic PBSC transplantation from an HLA-identical unrelated donor in Israel 3 months after achieving CR with chemotherapy (7+3). A myeloablative conditioning consisted of busulfan and cyclophosphomide, cyclosporin and methotrexate for the prophylaxis of GVHD were used. Limited stage acute GVHD with skin involvement was the only complication in the early post-HCT period. Six months later she presented with chronic GVHD with skin involvement that was controlled by tacrolimus and four rituximab infusions. 18 months post HSCT she appeared in our clinic with anasarca, lower limbs edema, hypoalbuminemia (21 g/L), massive proteinuria (increased from 3.5 to 37 g/day during a week). There was mild hematuria and normal renal function with no evidence of other autoimmune disease due to immunological tests. Ultrasound showed normal-sized and normoechoic kidneys, no hydronephrosis. A renal biopsy showed membranous glomerulonephritis (MGN). According to these findings, the diagnosis of MGN was established. We immediately restarted tacrolimus 0.5 mg/kg/day, prednisone 2-10 mg/kg/day and enalapril (10 mg/day) with no effect. In this case we decided to use rituximab in the standard dosage 375 mg/m$^2$ on days 1, 8, 15, 22. The edema resolved with furosemide and albumin transfusions. A month after the last infusion of rituximab she was asymptomatic with normal urine test, serum albumin was 38 g/L and renal function remained stable.Conclusion. Treatment of kidney cGVHD with rituximab is effective and well tolerated.

**P1068**
**Salvage therapy with everolimus improves quality of life in patients with refractory chronic graft-versus-host disease**
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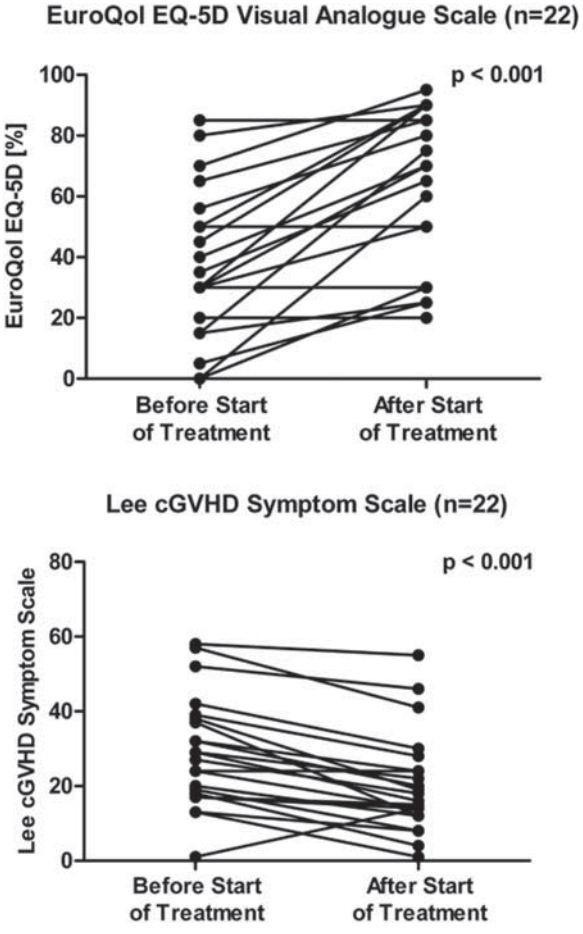
Objectives: Among many complications of allogeneic hematopoietic stem cell transplantation, chronic graft-versus-host disease (cGVHD) still remains one of the most important causes of impaired quality of life (QoL). Appearance of cGVHD usually requires long-term immunosuppression with steroids

**[P1068]**



and ciclosporin. The introduction of inhibitors of mammalian target of rapamycin (mTOR) has widened treatment options significantly. Here, we report our experience with Everolimus, a novel mTOR inhibitor, in patients with treatment-refractory cGVHD.
Methods: We treated 31 patients with a median age of 57 (19-70) years suffering from refractory cGVHD with Everolimus. We assessed potential toxicities and the response to treatment using Common Toxicity Criteria (CTC) and cGVHD Severity Score according to NIH Consensus Criteria as well as Everolimus plasma levels in a retrospective manner using patients' records. After written informed consent, we also performed actual and retrospective assessments of the subjective quality of life (EuroQol EQ-5D questionnaire) and of the degree of bother experienced by cGVHD symptoms (Lee Scale) in 22 out of these 31 patients. Nine patients were not assessable due to death (n=4), inability to comply (n=4), or refused participation (n=1).
Results: Thirty-one patients were treated with an average daily dose of 1.1 (0.5-3.5) mg Everolimus for a median duration of 320 (44-819) days. Average plasma levels were ranging from 1.4 to 9.2 ng/mL. Most frequent observed grade 3/4 CTC toxicities were infections (n=16) and thrombocytopenia (n=6). Four patients died of infectious causes, three of whom were still on Everolimus treatment. No disease relapse occurred. Regarding the objective response to treatment, 12 patients improved, 17 remained formally stable, and 2 worsened. However, 17 of 22 patients showed an improved QoL according to EQ-5D Visual Analogue Scale (37.5% *vs*. 70.0%; p<0.001) and a decline in the median Lee Scale was noted in 20 of 22 patients (28 *vs*. 17; p<0.001).
Conclusion: In this retrospective analysis, we found the majority of our patients to benefit from Everolimus salvage therapy as shown by the significant improvement of QoL and decline of bother even in cases where the formal cGVHD assessment suggested stability. The observed potential toxicity profile highlights infectious complications and myelotoxicity as a main issue while disease control does not appear to be impaired. Finally, these promising observations need to be verified in a prospective clinical trial.

**P1069**

**Recurrent oeosinophilic fasciitis on different locations of the body 3 years after allogeneic human stem cell transplanation**

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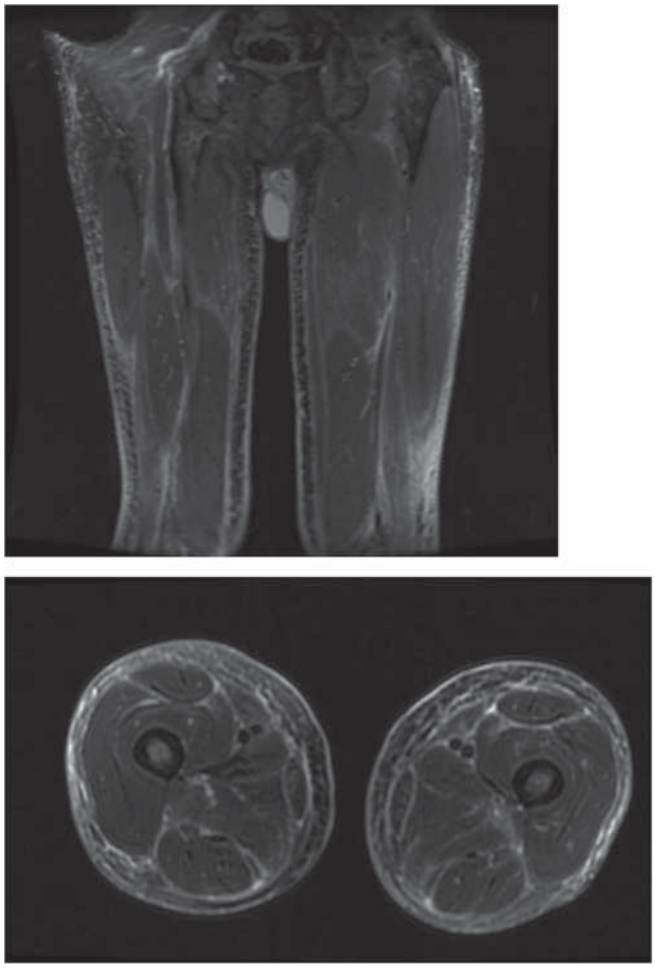
We present a 61 year old patient who received an allogeneic peripheral blood stem cell transplant from an HLA-identical sibling, following reduced conditioning after secondary osteo-

**[P1069]**



myelofibrosis. He developed painful swelling of both forearms three years post transplant. Peripheral blood showed borderline eosinophilia without inflammatory signs. By MRI the clinical diagnosis of eosinophilic fasciitis was confirmed, showing thickening of deep tissue fascia with profound signal intensification. The patient received 20 mg prednisone per day and the symptoms improved markedly so that steroid dosage was tapered and finally stopped 5 months later. After 1 month without immunosuppression he developed painful swelling with induration and profound redness of both thighs. He was hardly able to bend his knees. Peripheral blood showed neither eosinophilia nor inflammatory signs. Again MRI revealed oedema of the subcutaneous tissues as well as signal intensification of superficial and deep fascia seperating various muscles (Picture 1 and 2). Oedema and pain resolved rapidly after initiating steroids again.
The patient presented here illustrates the rare observation that eosinophilic fasciitis can reoccur on different sites of the body even after effective therapy.

**P1070**
**Vitamin B12 deficiency associated with three-month use of proton-pump inhibitor in a child with chronic graft-versus-host disease**
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Prolonged use of proton-pump inhibitors (PPI) could result in vitamin B12 deficiency as a consequence of impaired release of protein-bound dietary vitamin B12 from food in a nonacid environment. We describe a case of vitamin B12 deficiency associated with three months use of PPI in a patient with thalassemia major, during a chronic graft-versus-host disease (cGvHD) following hematopioetic stem cell transplantation (HSCT). A 12-year-old boy with thalassemia major underwent HSCT from his full matched 2 year old sister. His pretransplant vitamin B12 level was 202,6 pg/mL. Full engraftment was achieved, he was transfusion-independent with full single-donor chimerism and with good health condition until 13 months after the transplant. At that time he had presented with chronic cough and respiratory distress. He was diagnosed with cGvHD of the lungs. His vitamin B12 level was 345 pg/mL at that time. He received 2 mg/kg/day prednisolone and 1 mg/kg/day lansoprazole for three months until he had presented with paresthesia and weakness in his both arms and legs. Neurologic examination revealed normal except for a little sensation loss in his lower extremity. Spinal cord MRI and EMG findings were normal. Vitamin B12 deficiency because of prolonged use of PPI was suspected, his vitamin B12 level at the time was 94 pg/mL with macrocytosis and neutrophilic hypersegmentation on peripheral blood smear. Lansoprazole therapy was discontinued, he received parenteral 100 microgram/kg/week vitamin B12 for four weeks and all of his signs and symptoms resolved within that period. Malabsorption of dietary protein-bound vitamin B12 has been demonstrated with the prolonged use of PPIs. The malabsorption of dietary vitamin B(12) is thought to be a result of its impaired release from food protein, which requires gastric acid and pepsin as the initial step in the absorption process. It is recommended that patients taking these medications for extended periods of time, particularly >4 years, should be monitored for vitamin B12 status. This is the first case in the literature who developed vitamin B12 deficiency within a very short time of three months longstanding PPI therapy. The children with chronic graft versus host disease who are receiving prolonged PPI therapy should be closely monitored for vitamin B12 status independent of the therapy period.

**P1071**
**Autoimmune haemolytic anaemia in recipients of unrelated donor umbilical cord blood transplantation**
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Background: Immune-mediated hemolysis, allo/autoimmune, is a well-recognized complication after allogeneic hematopoietic stem cell transplantation (HSCT). The most common pathogenetic mechanism is incompatibility in red blood cell (RBC) antigens, mainly of the ABO system, between donor and recipient. However, autoimmune hemolytic anemia (AIHA) is a rarely recognized complication of HSCT, generally associated to chronic graft-versus-host disease (cGVHD). We report two cases of AIHA in a cohort of HSCT recipients from unrelated donor umbilical cord blood (UCB).
Methods and Materials: From May 2006 to August 2011, 27 UCB transplantations (4/6 or 5/6 HLA matched) were performed by Our Centre (14 female, 13 male, median age 40 y, range 16-65). Diagnosis included 16 AML, 3 CML, 4 lymphoproliferative disease, one SAA, one ALL and one MDS. Six patients were transplanted with double UCB; six pts received a reduced intensity conditioning. GVHD prophylaxis consisted of cyclosporine + mycophenolate mofetil; 18 pts received also *in vivo* T depletion with antithymocyte globulin (ATG). AIHA occurred in two pts (both transplanted with a 4/6 HLA matched single cord blood unit after conditioning with Thiotepa, Fludarabine, Busulfan and ATG): patient one was a 53 y old woman, affected by AML M4FAB, relapsed after autoHSCT. She developed at +235 cutaneous late onset acuteGVHD with atypical features, partially responding to steroids and extracorporeal photoapheresis. At +630, she manifested AIHA (Hb 4.7 g/dl, direct and indirect Coombs test +) resistant to immunoglobulin, methylprednisolone, and rituximab. Thereafter, she was treated with rituximab, cyclophosphamide, vincristine and prednisone, with improved level of hemoglobin at 4 months of follow-up. The second case was a 16 years old boy, affected by AML M2FAB, FLT3neg, t(6;9). He was transplanted without complications and developed AIHA at +115 days (Hb 5.5 g/dl, direct and indirect Coombs test+), resistant immunoglobulin, methylprednisolone, rituximab, cyclophosphamide and splenectomy. Therefore, he was treated by rituximab 100 mg/weekly and alemtuzumab 10 mg for 3 consecutive days with improved level of hemoglobin after 6 months of follow-up.
Conclusions: AIHA is a rare complications after HSCT of adults recipients specially with CB graft, probably related to posttransplant immunosuppression inducing an immune dysregulation and graft directed cell destruction. We report two cases of AIHA steroids refractory in a cohort of 27 UCB HSCT adults recipients.

## Reduced-intensity Conditioning

**P1072**
**FLAMSA-RIC for haematopoietic cell transplantation can overcome the poor prognosis of primary refractory or relapsed AML: a single-centre experience**
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Patients with relapsed or primary refractory acute myeloid leukemia (AML) have a dismal prognosis even after allogeneic hematopoietic cell transplantation (HCT). Recently, promising results have been reported using a sequential strategy with

aplasia inducing chemotherapy consisting of Fludarabine, Ara-C and Amsacrine (FLAMSA) followed within 3 days by reduced intensity conditioning (RIC) for allogeneic HCT (Schmid *et al.*, 2006 Aug 1;108(3):1092-9).
We report our experience with FLAMSA-RIC in primary refractory or relapsed AML patients. From 2006-2001 53 patients (f=23, m=30) were transplanted after FLAMSA-RIC. RIC consisted of fludarabine/busulfan (Flu/Bu, n=10), total body irradiation 4 Gy/cyclophosphamide (TBI 4Gy/Cy, n=28) or busulfan/cyclophosphamide (Bu/Cy, n=15). Median age of patients was 55 (range, 20-72) years. Patients were refractory after chemotherapy (n=23) or had untreated relapse (n=30). As GVHD prophylaxis calcineurin inhibitor combined with mycophenolate mofetil and anti-thymocyte globulin was used. 11 patients were transplanted from matched related donors (MRD), 17 from matched unrelated donors (MUD), 21 from mismatched unrelated and 4 from mismatched related donors. Current overall survival (OS) was 19/53 patients with a median follow-up of 497 days (range, 104-1203) resulting in a Kaplan-Meier estimated 2-year OS of 38%. OS in respect to the different RIC regimens was 56% with Flu/Bu *vs.* 29% with TBI 4Gy/Cy and 45% with Bu/Cy (p=0.28). Incidence of acute graft-versus host disease (GVHD) ≥II and chronic GVHD was 21% and 28%, respectively. Causes of death were relapse (n=20), infections (n=5), GVHD (n=2), multi-organ-failure (n=5) and others (n=2). Cumulative incidence of relapse at 2 years was 39% and of non-relapse mortality 25%. The outcome in the elderly subgroup defined by age ≥60 years (median age 67, n=22) was similar to the group of younger patients (median age 46, n=31) with 2-year OS of 35% *vs.* 40% (p=0.83). Patients with a blast count <10% at time of HCT had a better outcome with 67% *vs.* 29% OS (p=0.1). 2-year-OS was inferior in refractory compared to relapsed patients (33% *vs* 41%, p=0.73).
Allogeneic HCT using FLAMSA-RIC enables long-term disease free survival, even in primary refractory or relapsed AML patients. The sequential approach of this regimen seems to overcome the dismal prognosis of these patients. Its moderate toxicity allows the application of this curative salvage therapy option even in elderly patients.

**P1073**

**Outcome of allografting after toxicity-reduced conditioning in patients aged 60 years or older with haematological malignancies – a single-centre experience**

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The introduction of toxicity-reduced conditioning opened up the vista of allogeneic stem cell transplantation (allo-SCT) also for elderly patients (pts), aged 60y or more. However, up to date published evidence on the outcome of allo-SCT in this pt cohort is fairly limited by small pt numbers and/or short follow-up. This report summarizes our cumulative experience in a cohort of 163 consecutive elderly pts (age 60-73, median 63y) allografted from 2000 to 2010. Diagnoses were acute leukemia with active disease (group 1: induction failure n=25, untreated relapse n=22, refractory relapse n=12, RAEB n=25, CML-BC n=2), high risk acute leukemia in CR (group 2: n=28), and chronic diseases (group 3: mpn n=23, mds/mpn n=7, rcmd n=4, myeloma n=9, low grade B-cell neoplasm n=6). All pts received toxicity-reduced conditioning. For group 1 and 2 most often FLAMSA-RIC was applied, whereas in group 3 protocols based on intravenous busulfan or melphalan combined with fludarabine were utilized in the majority of pts. 25% of pts were grafted from a sibling donor and 75% from a matched unrelated donor (MUD). Stem cell source were peripheral blood stem cells in most cases. Eligible pts (being alive and free of leukaemia at day +120) conditioned with the FLAMSA-RIC protocol also received adjuvant donor lymphocyte infusions. After a median follow-up of more than 3y (range 0.6–11y) for surviving pts the Kaplan-Meyer procedure estimates a 38% probability of survival at 5y after transplantation for the whole cohort. When analyzing the different disease groups probability of survival at 5y was best in group 3 with 50%, followed by 47% in group 2 and 30% in group 1. Relapse occurred in 23% of group 1 pts and 7% in group 2 pts and was fatal in these 2 groups for all but 1 pt currently receiving irradiation for extramedullary relapse. In group 3 16 pts relapsed, however, 6 of these pts could be salvaged either by chemotherapy or second allotransplantation. Non-relapse mortality (NRM) for the whole cohort was 19% at day +100 and 38% during the entire observation period. NRM was highest in group 2 (46%), followed by group 1 (42%), and lowest in group 3 (27%). However, these differences were not significant. No difference in survival was observed in MUD or sibling transplants. Our data support the notion that toxicity-reduced conditioning followed by allo-SCT from sibling donors or MUD can be applied in elderly pts with high-risk disease and has excellent anti-leukemic efficacy. However, more efforts are needed to further reduce NRM.

**P1074**

**Allogeneic stem-cell transplantation in the late 7th and 8th decade of life; co-morbidity score and disease status but not age alone correlate with outcome**

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Reduced intensity conditioning (RIC) allows SCT in elderly patients (pts), not eligible for myeloablation. However, there is still paucity of data on the expected outcome and prognostic factors in the late 7th and 8th decade of life. We retrospectively analyzed SCT outcomes in 90 pts older than 65 years given allogeneic SCT in a single institution between the years 2001 and 2011. Median age was 68 years (65-76), 24 were age >70. Diagnoses included AML (n=45), MDS (n=21), lymphatic malignancies (n=19) or others (n=5). 21, 19 and 50 pts had early, intermediate and advanced disease status at SCT. 20 pts had a comorbidity score (HCT-CI) >2. The donor was sibling (n=47), matched unrelated (n=40), mismatched related (n=2) or cord blood (n=1). The conditioning regimen was RIC (n=54) or a more intensive reduced toxicity myeloablative conditioning (RTC, n=36). 84 pts engrafted in a median of 14 days, 5 died prior to engraftment and one had primary graft failure. With a median follow-up of 15 months (2-120), 27 are alive; 63 died of relapse (n=33) or non-relapse causes (NRM, n=30). The cumulative incidence of NRM was 16%, 28% and 31% at day +100, 1 and 2 years after SCT, respectively and was not different among pts age 65-69 and pts age >70 years. The only predicting factors for NRM in multivariate analysis (MVA) were HCT-CI >2 (HR 4.0, p=0.01) and advanced disease status (HR 4.6, p=0.008). Acute GVHD occurred in 30 pts, and was associated with 13 deaths (16% of all pts, all 13 pts with Gr III-IV acute GVHD subsequently died). The 2-year cumulative incidence of relapse was 39% with advanced disease been the most significant predicting factor (HR 5.0, p=0.002). The 2-year overall survival (OS) of the entire group was 37%. It was 34% and 37% for pts age 65-69 and >70 years, respectively (p=NS). 2-year OS was 57%, 37% and 28% in pts with early, intermediate and advanced disease status at SCT, respectively (p=0.006) and 40% and 23% for pts with HCT-CI >2 or 0-2, respectively (p=0.2). In MVA these were the only predictive factors with HR 3.0 (p=0.003) and 2.1 (p=0.03), respectively. The 2-year OS of the 28 pts with no comorbidities and no advanced disease was 51%. Age alone, donor or disease type were not predicting for outcome. In conclusion, allogeneic SCT is feasible in a subset of older pts in the late 7th and 8th decade of life with reasonable outcome, if they have no significant comorbidities and no advanced disease status, when using RIC and RTC conditioning.

**P1075**

**Impact of acute renal failure in reduced-intensity conditioning allogenic stem cell transplantation**

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Objectives: Acute renal failure (ARF) in allogeneic stem cell transplantation has a multifactorial etiology and can compromise the procedure results. There is scarce information on the prognostic significance of ARF in the setting of reduced-intensity conditioning allogenic stem cell transplantation (RIC-SCT). The objective of this study was to analyze the incidence and impact of ARF in pts. receiving allogenic RIC-SCT.

Method: Between 2001 and 2010, 73 RIC-SCT were performed in a single center (62% males, median age 55 [27-69]) Fifty-six out of 73 pts. (77%) developed ARF at some point between the days -7 and 360 after transplantation. Cyclosporine (CyA) dosage was adjusted 3 times a week during the admission time. ARF severity was divided in 4 grades, according to the formula MDRD-4 IDMS for glomerular filtration (GF), defining 5 stages (0-2: >60 mL/min; 3: 30-59 mL/min; 4: 15-29 mL/min; 5: <15 mL/min). Six pts. had a stage 3 renal failure before RIC-SCT.

Results: The underlying disease was acute leukemia in 28 pts. (38%), low-grade lymphoproliferative disease in 13 (18%), high grade lymphoma in 17 (23%), myelodysplastic syndrome in 7 (10%), multiple myeloma in 5 (7%) and chronic myeloproliferative syndrome in 3 (4%). Forty-six pts. (63%) received <3 treatment lines before the SCT, and the remaining ≥ 3 lines. Conditioning regimens were: mephalan plus fludarabine (n=34, 52%), busulfan plus fludarabine (n=25, 38%) and high dose melphalan (n=7, 10%). RIC-SCT was non-related in 10 pts. (14%). The median follow-up was 32 months (0.5-112). Previous comorbidities were: hypertension (13), diabetes mellitus (7) and hearth failure (1). The majority of ARF developed before day +30 after SCT. Seventeen patients (23%) were in stage 0-2, 32 pts. (44%) presented stage 3 ARF, 22 (30%) stage 4 ARF, and 2 pts. (3%) stage 5 ARF. In this series, ARF was not associated with high levels of CyA on days 15, 30, 60, 120 and 360 after the infusion. We neither found an association with a previous history of diabetes, hypertension or acute or chronic graft-versus-host disease. The overall survival probability at 1-year was significantly lower in patients with stage 4-5 compared to those in stage 0-3 (24% ± 18% *vs.* 56% ± 15%, p=0.01).

Conclusions: ARF is a frequent complication after a RIC-SCT, having a negative effect on pts. survival. None of the comorbidities before RIC-SCT was associated with increased frequency of ARF.

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**P1076**

**Comparison of early toxicity, transplantation-related mortality and relapse mortality after two different RIC regimens in myeloid malignancies: fludarabin/treosulfan and FLAMSA-RIC**

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Objectives: Allogeneic Stem Cell Transplantation (alloSCT) is a curative therapy for patients with advanced myeloid malignancies. Elderly or heavily pretreated patients often receive Reduced Intensity Conditioning (RIC) regimens. The combination of Fludarabin/Treosulfan (Flu/Treo) is a relatively intensive RIC-regimen with myeloablative potential often used in elderly patients with AML or MDS. For patients with refractory myeloid malignancies the FLAMSA-RIC protocol was established to overcome resistance to chemotherapy. We analyzed the two protocols with regard to early toxicity, Transplantation Related Mortality (TRM), Non Relapse mortality (NRM), Relapse Mortality, and Overall Survival (OS).

Methods: Single center retrospective analysis of two patient cohorts. Last follow up was on Dec. 1st 2011. Median follow up was 20.1 month for Flu/Treo and 15.7 month for Flamsa-RIC.

Cohort 1: Flu/Treo (n=30). Fludarabin 30 mg/m2 d-6 to -2 and treosulfan 14 g/m$^2$ d-6 to -4. Three patients received bone marrow (BM), 25 patients peripheral blood stem cells (PBSC) and two patients BM and PBSC. Median age was 58 years, male: female ratio 18:12, (s)AML (CR1: n=10, CR2 n=2, advanced disease n=8), MDS (n=2), MPS (n=3), CML-CP (n=1) CML-AP (n=1), CML-BC (n=3), MUD:MRD 19:11, female donor -> male patient: n=8. EBMT-risk score (median): 4.3.

Cohort 2: FLAMSA-RIC (n=28). Fludarabin 30 mg/m$^2$, cytarabin 2 g/m$^2$, and amsacrine 100 mg/m2 d-12 to -9, busulvan i.v. 0.8 mg 4x/day d-5 to -4, and cyclophosphamide 60 mg/kg d-3 to -2. 27 patients received PBSC and one patient double umbilical cord blood. Median age was 49y, male:female 15:13, (s)AML (CR1 n=2, advanced disease n=18), MDS (n=4), MPS (n=2), CML-BC (n=2), MUD:MRD 22/6, female donor -> male patient: n=6. EBMT-risk score (median): 4.7.

GVHD prophylaxis was identical in the two cohorts and consisted of cyclosporin-A and mycophenolate mofetil. All patients with a matched unrelated donor additionally received ATG Fresenius 20 mg/kg on d-3 to -1. Supportive care was also identical in the two cohorts.

Results are summarized in Table 1.

**[P1076]**

| | Flu/Treo (n=30) | FLAMSA-RIC (n=28) |
|---|---|---|
| Hospital stay (days) | 44 (median, range: 21-145) | 48 (median, range: 18-140) |
| Duration of Neutropenia (ANC<500/µl, days) | 21(median, range: 13-29) | 27 (median, range: 19-34) |
| Duration of fever (days) | 5 (median, range: 0-16) | 11 (median, range: 3-64) |
| Duration of antibiotic therapy (days, median) | 20 (median, range: 6-42) | 31(median, range: 9-101) |
| No. of packed red cells | 10 (median, range: 2-25) | 11 (median, range: 0-45) |
| No. of Thrombocyte transfusions | 14 (median, range: 3-38) | 20 (median, range: 1-89) |
| Engraftment ANC >500/µl | 20 (median, range: 14-28) | 21 (median, range: 13-48) |
| Engraftment Thr. >20G/l | 18 (median, range: 13-54) | 22 (median, range: 10-52) |
| Weight gain during conditioning | 6.52% | 6.86% |
| Increase of bilirubin during conditioning until day +28 | 4.4 fold (d+1) | 6.4 fold (d+4) |
| Increase of creatinin during conditioning until day +28 | 1.5 fold | 2.0 fold |
| TRM d30 | 6.7% | 10.3% |
| TRM d100 | 6.7% | 20.7% |
| TRM d365 | 24% | 28% |
| NRM (at last follow up) | 6.7% | 0% |
| Relapse Mortality(at last follow up) | 20% | 27.6% |
| OS (at last follow up) | 50% | 44.8% |

Conclusion: The conditioning with Flu-Treo shows little early toxicity after alloSCT. It compares favorably with FLAMSA-RIC conditioning. However, the patient cohorts differed in many characteristics. Based on these data, a prospective and randomized study is justified to compare the outcome of the different conditioning regimens.

**P1077**

**Allogeneic haematopoietic stem cell translantation following reduced-intensity conditioning in patients with haematological malingancies: what is the role of anti-thymocyte globulin (ATG)?**

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The frequency of allogeneic hematopoietic stem cell transplantation with reduced intensity conditioning (RIC-allo HSCT) is steadily increasing the last few years reaching nowadays approximately 40% of all transplantations. ATG is thought to facilitate engraftment and GVHD prevention although no randomized clinical trials have been conducted to test this. We retrospectively analyzed (11/1999-12/2010) the impact of ATG on the outcome of RIC-allo HSCT in 49 patients [31M, 18F, Deltam 52 years (14-63)] with various hematological malignancies who received preparative regimens containing fludarabine and an alkylating agent either along with ATG - 7,5 mg/kg (group A, n=28)- or without (group B, n=21). Most patients had unfavorable prognostic factors at diagnosis with no major differences between groups A and B in their baseline characteristics (33/49) being submitted to transplantation at an advanced stage of disease (29/49). Hematopoietic cell transplantation comorbidity index (HCT-CI) was ≥2 in 22/49 patients being similar in the 2 groups. 7/28 (25%) and 11/21 (52%) developed acute GvHD (gr II-IV) from groups Alpha and Beta respectively, while 3 patients from each group succumbed due to gr IV aGvHD. Chronic GvHD was observed in 13/23 (56%) evaluated patients from group A and in 14/18 (78%) from group Beta (1 and 3 patients succumbed, respectively). Infections did not differ between the 2 groups neither during the first 100 days nor later. Within a Deltam time from transplantation 10 (1-102) months, 15/20 (75%) evaluated patients from group A were refractory or relapsed (11 deaths) while from group B the respective percentage was 42% (4 deaths). The administration of ATG seemed to negatively influence overall survival (OS) [estimated 5year OS for groups A and B: 12% versus 41% respectively, (p=0,07)] while it did not affect Treatment Related Mortality (TRM) and Relapse Rate (RR), perhaps due to the small number of patients studied. Moreover, HCT-CI≥ 2 had a negative impact on OS, TRM and RR (p<0,05), while the status of the disease was an adverse prognostic factor for RR (p<0,05).
In conclusion, the results from this retrospective study may suggest that the a priori administration of ATG on RIC-allo HSCT should be reconsidered and may be limited to unrelated donor transplantations or to cases of incompatibility between donor and recipient.

**P1078**

**Acute and chronic GvHD after reduced-intensity conditioning umbilical cord blood transplantation in adults with haematological malignancies: evaluation of NIH criteria in a retrospective single-centre analysis**

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Graft versus host disease (GVHD) remains a major concern affecting morbidity and mortality after AHSCT. We retrospectively reviewed 71 consecutive adult patients who underwent UCBT after reduced intensity conditioning regimen at our center to evaluate the incidence and severity of acute and chronic GVHD according to Seattle (classic) criteria and NIH consensus criteria, recently described.
Cumulative Incidence (CI) of classic acute GVHD grade II-IV, grade III-IVand NIH acute GVHD were 0,34; 0,18 and 0.38 respectively. CI of Classic and NIH Chronic GVHDat 1-year were 0,35 and 0,29 respectively. There was no severe NIH cGVHD following UCBT. Overall response to immunosuppressive therapy for aGVHD and cGVHD were 89% and 100% respectively. OS and PFS at 3-years were 47% and 40% respectively. There was no impact of aGVHD on survival.
We conclude NIH criteria are relevant for assessing GVHD following UCBT. Future clinical studies are warranted to evaluate prospectively GVHD through NIH criteria. These may allow a better understanding of biological mechanisms of GVHD following UCBT to establish adequate therapeutic strategy.

**P1079**

**Haploidentical non-myeloablative stem cell transplantation with high-dose post-transplant cyclophosphamide using unmanipolated mobilized peripheral stem cells: fast engraftment, no evidence of graft rejection and low incidence of GvHD**

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Haploidentical bone marrow transplantation using non-myeloablative conditioning and high-dose, post-transplant cyclophosphamide (Cy) has been shown to be a feasible approach for patients lacking an HLA identical donor with acceptable rates of acute and chronic Graft-versus-Host-Disease (GVHD); in the attempt to reduce the associated risk of graft failure and relapse incidence we decided to apply the same conditioning regimen using unmanipolated mobilized peripheral blood stem cells (PBMSC). From April 2010 to November 2011, 10 patients, median age 54 (21-70), affected by high-risk hematological malignancies (4 acute myeloid leukemia, 3 lymphoblastic leukemia, 2 Hodgkin's Lymphoma, 1 myelodysplastic syndrome) and with no available HLA identical donor neither related or unrelated received the following conditioning regimen: Cy 14.5 mg/kg/day iv on days -6 and -5, fludarabine 30 mg/m$^2$/day iv on days -6 to -2, and 200 cGy of TBI on day -1. On day 0, patients received a median of 5.9x10$^6$ (4.4-8.8) CD34+ cells/kg with a median of CD3+ of 2.5x10$^8$/kg (1.3-4.7). On day +3 and +4 Cy 50 mg/kg was administered. From Day +5 patients received tacrolimus daily which was tapered off by day +180. Micofenolate Mofetile was given until day +35, 15 mg/kg orally tid. Patients received G-CSF 5 mg/kg/day from day +5 and continuing until ANC ≥500/uL for 3 days. Disease status at transplant was the following: 3 progressive disease (PD), 1 SD, 5 complete remission (CR), 1 partial response. At a median follow-up of 152 days (42-463), OS is 60% with 3 patients in CR and 3 in PD.
Grade I/II acute GVHD occurred in 2 patients (20%). No cGvHD was observed in 7 of 10 evaluable patients with a median time follow-up of 216 days (131-463). CMV reactivation occurred in 5/10 patients, resolved with preemptive therapy in 4 and caused a fatal infection in 1 heavily pretreated patient, with a cumulative non relapse mortality of 10%. Notably, no graft failure was observed. Achievement of full donor chimerism was rapid and complete on CD3+ cells in 7/10 evaluable patients by day +84. Our data show that haploidentical non-myeloablative PBMSC transplantation with high-dose post-transplant Cy is a feasible and safe approach for patients lacking an HLA identical donor. The use of unmanipulated PBMSC with the infusion of a greater number of CD3+ cells allows a rapid and sustained engraftment, reduces the risk of graft failure and seems not to increase the risk of GVHD.

**P1080**

**A prospective randomised study comparing reduced-intensity conditioning and myeloablative conditioning in patients with myeloid leukaemia undergoing allogeneic haematopoietic stem cell transplantation**

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There have been no randomised studies comparing myeloablative conditioning (MAC) and reduced-intensity conditioning (RIC) in allogeneic haematopoietic stem cell transplantation (HSCT). We wanted to compare the safety and efficacy of these two regimens.
We performed an open-labeled, randomised, controlled phase-III trial. Over a 10-year period, adult patients ≤60 years of age with myeloid leukaemia were randomised (1:1) to undergo RIC (n=18) or MAC (n=19). We included recipients of HLA-A, HLA-B, or HLA-DRB1-identical unmanipulated grafts from related or unrelated donors. The primary endpoint was transplant-related mortality (TRM). Secondary endpoints included relapse, survival, chimerism, and toxicity.
The RIC patients had faster platelet engraftment ($p<0.01$), required fewer erythrocyte and platelet transfusions ($p<0.001$), and required less total parenteral nutrition (TPN) than the MAC group ($p<0.01$). Cytomegalovirus reactivation was commoner in the MAC group (14/19) than in the RIC group (6/18) ($p=0.02$). Haemorrhagic cystitis occurred in eight of the MAC patients and in none of the RIC patients ($p<0.01$). Donor chimerism was similar in the two groups regarding CD19 and CD33, but was delayed for CD3 in the RIC group. Later chimerism status was similar. Incidences of acute and chronic graft-versus-host disease (GVHD) were similar in the two groups, but two MAC patients and no RIC patients died of GVHD. Five-year TRM was around 11% in both groups, and relapse and survival were not significantly different. The MAC patients with intermediate cytogenetic acute myeloid leukaemia had a three-year survival of 73%, as compared to 90% in the RIC patients.
TRM was low with RIC and MAC. RIC had several advantages such as faster platelet engraftment, fewer transfusions, less TPN, fewer CMV reactivations, and less haemorrhagic cystitis.

**P1081**

**Long-term outcome of patients with acute myeloid leukaemia and myelodysplastic syndrome after reduced-intensity conditioning for allogeneic haematopoietic cell transplantation**

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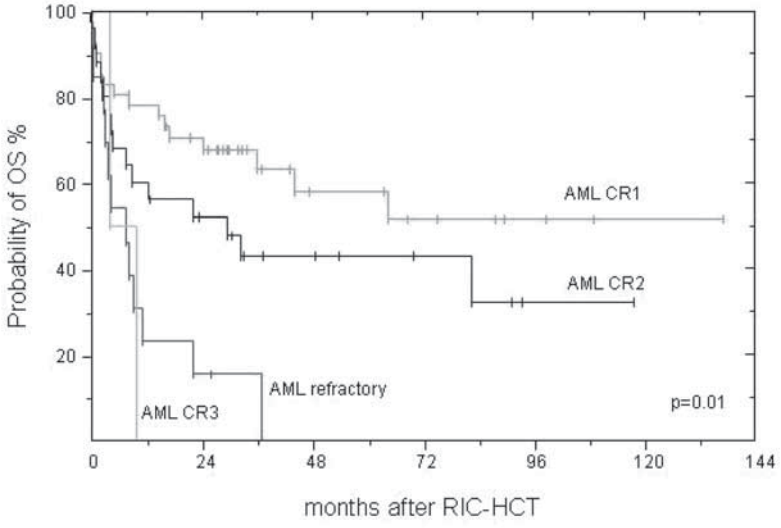
Allogeneic hematopoietic cell transplantation (HCT) is an effective treatment for patients with acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS). Reduced-intensity conditioning (RIC) regimens have been developed with the aim to perform safer allogeneic HCT in patient populations previously discarded because of higher HCT-related mortality.
Between April 1999 and December 2009, a total of 95 consecutive patients (44 male, 51 female) with de novo AML (n=62), secondary AML (n=23) and MDS (n=10) underwent reduced-intensity conditioning (RIC) followed by allogeneic HCT. RIC consisted of fludarabine/total body irradiation of 2 Gy according to the Seattle protocol in 21 patients (22%), the FLAMSA protocol in 70 (74%) and other chemotherapy regimens in 4 patients (4%). All patients were ineligible for myeloablative HCT because of age or comorbidities. Donors were siblings in 31 (33%) patients and unrelated (URD) in 64 (67%) patients. The majority of patients received granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood stem cells (n=91, 96%). Graft-versus-host disease prophylaxis consisted of cyclosporine A and mycophenolate mofetil in the majority of patients.
With a median follow-up of 37 (range, 6-137) months, 39 patients (41%) are alive. Overall survival (OS) and disease free survival (DFS) rates at 5 years for the whole cohort were 40% and 57%, respectively. OS and DFS at 5 years for patients with AML was 43% and 56%, for patients with MDS 20% and 71%, respectively. The cumulative incidence of non-relapse mortality at 5 years was 30% and was significantly higher for patients with MDS (70%) than for patients with AML (24%; $p<0.01$). No difference in OS and DFS projected at 5-years could be observed comparing different RIC protocols (Seattle 41% and 45% *vs.* FLAMSA 42% and 50%) or donor type (Sibling 43% and 38% *vs.* URD 41% and 60%). Patients with AML had a significantly ($p=0.01$) better OS when transplanted in 1st or 2nd complete

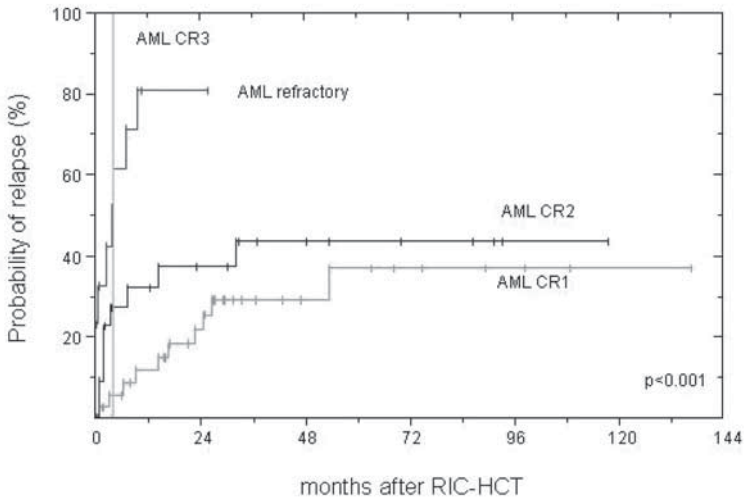
**[P1081]**

Figure 1: Overall Survival for AML and secondary AML



[P1081]

Figure 2: Risk for relapse for AML and secondary AML




remission (CR) than in 3rd CR or with refractory disease (Figure 1). Additionally, patients with AML in 3rd CR or with refractory disease had a significantly (p<0.001) higher risk for relapse than patients transplanted in 1st or 2nd CR (Figure 2).
RIC followed by allogeneic HCT is an effective therapy for patients with AML in 1st and 2nd remission at time of transplant. For patients with refractory disease and MDS investigation of less toxic but efficient novel regimens are warranted.

**P1082**

**Potent graft-versus-leukaemia effect after reduced-intensity allogeneic stem-cell transplantation as post-remission therapy for intermediate-risk de-novo AML with FLT3-ITD genotype or wild-type (WT) NPM1 and CEBPA without FLT3-ITD**

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Mutational status of FLT3, NPM1 and CEBPA is associated with the outcome in intermediate-risk AML (Schlenk, NEJM 2008). Benefit of myeloablative HLA-matched related ASCT is limited to pts with FLT3-ITD or genotype consisting of WT NPM1 and CEBPA without FLT3-ITD (triple neg) and provided a better RFS not translating into a better OS. To investigate the role of reduced-intensity allogeneic SCT (RIC-ASCT) as post-remission therapy in adult FLT3-ITD or triple neg intermediate-risk AML pts as post-CR1 therapy, we conducted a single center retrospective analysis.
Methods: pts aged 18 up to 65 diagnosed with FLT3-ITD or Triple neg de novo AML between Jan 2001 and Dec 2010 in CR1 were reviewed, excluding secondary AML, APL, AML with favorable or unfavorable karyotypes (according to Döhner, Blood 2010). Pts excluded from ASCT because of a poor performance status were excluded as were pts deceased before the median time between CR1 and ASCT. The only reason for not performing ASCT was the absence of a suitable donor.
Results: 67 pts were included, 37 treated with ASCT the "ASCT" group and 30 treated with non-ASCT therapies, the "no-ASCT" group. Both groups were comparable with respect to med age at dg, WBC at dg, sex ratio, proportion of normal/abnormal karyotype, or FLT3-ITD/triple neg genotype, and nb of chemotherapy to reach CR1. Med time between CR1 and ASCT was 114 days (24-295). Conditioning were fludarabine+busulfan+ATG, fludarabine+cyclophosphamide+TBI2Gy, fludarabine+TBI2Gy, fludarabine+treosulfan+ATG (n=20/4/10/3). Source of stem

[P1082]

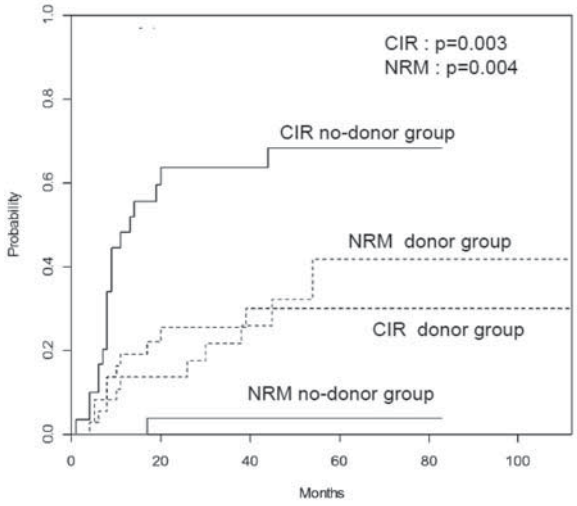


**Figure 3 CIR and NRM**
Univariate analyses indicated that CIR was reduced only by ASCT in CR1. Multivariate analysis for CIR indicated that ASCT was associated with a reduced risk of relapse (HR, 0.31; p=0,006). and a higher risk of NRM (HR=9.9; 95% CI [1.22-80] p=0.03).

**[P1082]**

| | Donor group n=37 | No-donor group n=30 | p |
|---|---|---|---|
| Year of diagnosis | | | 0.001 |
| 2001-2005 | 7 | 18 | |
| 2006-2010 | 30 | 12 | |
| Age at diagnosis, years | | | NS |
| Median (range) | 56 (31-64) | 54 (19-64) | |
| < 55 | 16 | 17 | |
| ≥ 55 | 21 | 13 | |
| Gender | | | |
| Male | 22 | 12 | NS |
| Female | 15 | 18 | |
| WBC > 25,000/µL at diagnosis | 11/37 | 12/30 | NS |
| Karyotype | | | |
| Normal | 22 | 21 | NS |
| Abnormal | 15 | 9 | |
| Genotype | | | |
| FLT3-ITD | 11 | 14 | NS |
| Triple negative | 26 | 16 | |
| Courses of chemotherapy to reach CR1 (n) | | | NS |
| 1 | 22 | 21 | |
| 2 | 12 | 9 | |
| 3 | 3 | 0 | |

cells was PBSC (n=32), BM (n=1), UCB (n=4). Donors were matched-related (n=18), matched-unrelated (n=11), mismatch-unrelated (n=8). In the ASCT *vs* no-ASCT groups, med F.U after CR1 was 37 months (11 to 112) *vs* 48 months (6-83) and 10 pts relapsed at a med time of 8 months (4-39) *vs* 19 pts at a med time of 8 months (1-44), respectively. In the ASCT *vs* no-ASCT groups, 3-y cumulative incidence of relapse (CIR) were 25% ± 7% *vs* 64% ± 9% (p=0.003), and 3-y NRM, OS, RFS were 21% ± 7% *vs* 4 % ± 4% (p=0.004), 52% ± 9% *vs* 42% ± 10% (p=0.88), and 53% ± 9% *vs* 34% ± 9% (p=0.2), respectively. At the last F.U, 19 pts have died (disease (n=9), infections (n=7), GvHD (n=2), suicide (n=1)) *vs* 15 pts (disease (n=14), infections (n=1)) in the ASCT *vs* no-ASCT groups.
Conclusion: RIC-ASCT decreases the risk of relapse as compared to conventional chemotherapy, suggesting a potent graft-versus-leukemia effect in these pts at a high-risk of relapse.

**P1083**
**Myeloablative and reduced-intensity conditioning for patients with MDS or MPS: a 10-year single-centre experience**
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Purpose: Hematopoetic cell transplantation (HCT) is the treatment of choice for many hematologic malignancies including myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPN). Nevertheless, not all patients tolerate myeloablative conditioning due to age and comorbidities. The aim of this retrospective study was to identify risk factors for outcome of patients with MDS and MPN receiving myeloablative (MAC) or reduced intensity conditioning (RIC) for HCT.
Patients and Methods: The outcome of 109 patients transplanted between 2000 and 2010 with a median age of 57 (range 18-70) years and a median follow up of 46 (range 1-119) months were analyzed. Patients were diagnosed with MDS (n=71), MPN (n=25) and MDS/MPN (n=13). A total of 34 patients received MAC-HCT, consisting of 12 Gy TBI (n=21) or 16 mg/kg Busulfan (n=13) in combination with 120 mg/kg Cyclophosphamide, while 75 patients were treated with low dose TBI (either 2 or 3 Gy; n=69) or 8 mg/kg Busulfan (n=6) combined with Fludarabine. Patients undergoing RIC-HCT were significantly older, had a higher EBMT risk score, more advanced diseases than patients in the MAC-HCT cohort.
Results: Estimated overall survival (OS) was 64% and 33% (p=0.05), event free survival (EFS) 64% and 27% (p=0.1) and relapse incidence (RI) 8% and 42% (p=0.1) at 6 years following MAC-HCT and RIC-HCT, respectively. There was no difference in non relapse mortality (NRM) between the two groups (p=0.35). Patients after RIC had a significantly lower incidence of acute graft versus host disease (GvHD; p=0.05), but not of chronic GvHD (p=0.28). In multivariate analysis testing age, EBMT score, lines of pre-treatment and GvHD severity there was no difference in OS for patients after RIC-HCT or MAC-HCT (p=0.11). However results confirm a lower EFS (p=0.05) and a trend towards higher RI (p=0.09) after RIC-HCT. As expected, there was a higher RI for patients with advanced disease, while combined acute (grade I and II) and chronic limited GvHD had an impact on better OS (p=0.001), better EFS (p=0.01) and lower NRM (p=0.03). Female gender affected favourably OS (p=0.03), NRM (p=0.02) and EFS (p=0.04).
Conclusion: Outcome for patients with MDS and MPN after RIC-HCT and MAC-HCT improved considerably during the last years. Despite older age and more advanced disease there was no difference in OS between MAC-HCT and RIC-HCT. There was only a trend towards higher RI after RIC-HCT in comparison to MAC-HCT. Low grade acute and limited chronic GvHD played a crucial role for OS, EFS and NRM.

**P1084**
**Fludarabine and busulfan as conditioning regimen in allogeneic stem cell transplantation: comparison with BuCy2**
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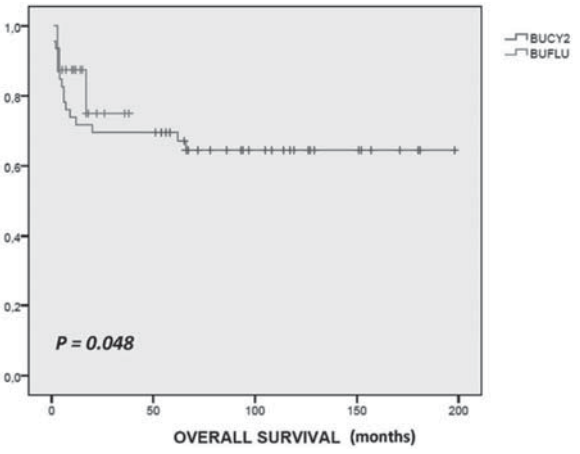
Background: Busulfan (Bu) and cyclophosphamide (Cy) are the most widely used myeloablative regimen to treat malignancies with allogeneic stem cell transplantation (alloSCT). Recent studies indicate that fludarabine (Flu) is less toxic than Cy but equally efficacy in immunosuppression and antileukemic activity.
Aim of the Study: To evaluate the efficacy of Flu compared to Cy in conditionings of alloSCT in patients (pts) affected by acute myelogenous leukemia.
Methods: We performed a retrospective analysis on 62 consecutive pts transplanted on our Unit from 1993 to 2011. 46 pts received BuCy2 and 16 pts BuFlu. 51 pts arrived to transplant in first complete remission (CR), 4 pts in 2nd CR, 6 pts in progression. Median (Mdn) follow up was 66.5 (range 0-198) and 14.5 months (r. 3-38) respectively (resp). Mdn age at transplant (41.5 years) was the same in both groups. Mdn time of hospitalization was 31 (r. 21-85) and 29.5 days (r. 24-42), resp. All pts receiving BuFlu had prognostic factors predictive of high risk of relapse. Those risk factors were not known in most of pts treated with BuCy2. Hematopoietic progenitor cells from apheresis were used as stem cell source (SCS) in 36 pts, bone marrow in 26 pts. In 27 pts Bu was administered per os and in 35 ev. GVHD prophylaxis was cyclosporine-A (CSA) with methotrexate (MTX) in 51 pts and with mycophenalate mofetil (MMF) in 12 pts. Mdn time of neutrophil recovery (>0.5x10E9/l)

and platelet recovery (>30 x 10E9/l) were 14 days (r.7-30 and r.5-155, resp).
Results: No significant statistical difference was documented between the efficacy of BuCy2 and BuFlu in terms of overall survival (OS) (P=0.48), event-free-survival (EFS) (P=0.29), transplant-related-mortality (P=0.27), disease related death (P=0.64), engraftment (P=0.55), immune recovery (P=0.48), acute GVHD (P=0.61), chronic GVHD (P=0.17). The impact of SCS on OS was the same into the 2 groups of pts (P=0.44). The efficacy on OS and EFS was the same when Bu was administered per os and ev (P=0.42 and P=0.78, resp) and when GVHD prophylaxis was perfomed with CYA+MTX or +MMF (P=0.33 and P=0.35, resp).
Conclusion: These data about the use of BuFlu in alloSCT are very encouraging. Considering that all the pts receiving BuFlu were at high risk of disease relapse and that survival curves were similar in BuFlu and BuCy2 groups in the first year of transplant when the incidence of relapse is higher, we think that these results will remain long after the transplant.




**P1085**
**Reduced-intensity conditioning with fludarabine, busulfan, and 4 Gy total body irradiation followed by allogeneic unrelated donor bone marrow transplantation**
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Background: Fludarabine-based reduced-intensity regimen (RIC) is widely used and shows good safety and efficacy. We retrospectively investigated the feasibility of RIC with fludarabine, busulfan and total body irradiation (TBI) prior to unrelated donor bone marrow transplantation (BMT).
Methods: We analyzed 30 adult patients (female 15, male 15) with AML (n=9), ALL (n=2), MDS (n=7), CML (n=1), Adult T cell leukemia/lymphoma(ATLL) (n=4), CMML (n=1), NHL (n=4), Hemophagocytic syndrome (n=1), Chronic active EBV infection (n=1).
Reduced-intensity preconditioning was performed with fludarabine (30 mg/m$^2$ for 6 days), busulfan (4 mg/kg po for 2 days) and 4 Gy TBI followed by allogeneic BMT from unrelated donors facilitated by Japan Marrow Donor Program from April 2003 to July 2007 in our institution. The median age at transplant was 53.5 years (range, 18-64).
Cyclosporine + Methotrexate (MTX) or tacrolimus + MTX were used as graft-versus-host disease (GVHD) prophylaxis in HLA allele matched or HLA allele mismatched patients, respectively.
The reason for choosing RIC were mainly age (over 50 years old) and concurrent morbidity.
Results: Engraftment was achieved in 27 patients at a median of day 17 (range, day 14-34) after transplantation. Grade II to IV acute GVHD and extensive chronic GVHD occured in 20 of 27 (74.1%) and 3 of 21 (14.3%) patients, respectively. Hepatic VOD occurred in 2 of 30 (6.7%) patients. With a median follow-up of 848 days (range, 12-3,039 days), actuarial overall survival rate was 46.2%.
16 patients died, and the causes of death were relapse or disease progression (n=6), pulmonary complications (n=5), TMA (n=2), Intestinal bleeding (n=1), Encephalitis (n=1) and Heart Failure (n=1). The remaining 14 patients (at transplantation, 8 were in remission, 3 (CML 1, ATLL 2) were not in remission and 3 were with MDS) are currently still in remission.
Conclusion: RIC with fludarabine, busulfan and 4GyTBI was feasible and tolerable for adults with high risk hematological diseases, but it is important that disease and disease status before BMT should be carefully determined.

**P1086**
**Fludarabine with cytarabine and sequential reduced-intensity conditioning with allogeneic stem cell transplantation in 15 patients with poor-risk chronic lymphocytic leukaemia**
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Background: Allogeneic stem cell transplantation (SCT) is a treatment option for younger patients (pts) with poor-risk chronic lymphocytic leukemia (CLL). Sequential use of chemotherapy and reduced-intensity conditioning (RIC) for SCT can increase therapeutic effect in high-risk leukemia pts, we used the similar protocol without amsacrine in CLL pts.
Methods: Poor-risk CLL was defined by one of the following: refractoriness or early relapse (within 12 months) after treatment with a purine analogue-containing regimen, progressive disease in the presence of an unfavorable genetic constellation (17p-, and/or unmutated IGHV status). Fludarabine (30 mg/m$^2$) and cytarabine (2 g/m$^2$) for 4 days (FC) were used for cytoreduction. After 3 days of rest, RIC consisting of 4 Gy TBI, anti-thymocyte globulin 10-20 mg/kg/day for 3 days, and cyclophosphamide 40-60 mg/kg/day for 2 days followed. We analyzed 15 pts with CLL undergoing chemotherapy and RIC SCT in our centre from August 2007 to June 2011.
Types of donors and used grafts were as follows: HLA identical sibling, n=6; unrelated donor, n=9, PBSCs, n=13, BM, n=2. Median age of pts was 50 years (range 38-61). Quantitative monitoring of minimal residual disease (MRD) after SCT was performed by four-colour flow cytometry and real time PCR.
Results: The median time of neutrophil engraftment (above 0.5x10E9/L) was 16 days, all pts engrafted. The most frequent toxicities were grade III/IV infections according to common toxicity criteria in 12 of 15 pts and gastrointestinal toxicities (grade III in 8 of 15 pts).
Incidence of acute GVHD was evaluated in 14 pts: 50% (7/14) of pts had GVHD (grade I+II in 4 pts, grade III in 3 pts). Incidence of chronic GVHD was evaluated in 13 pts, 46% (6/13) of pts had GVHD (limited in 4 pts, extensive in 2 pts).
Nonrelapse mortality (NRM) after 1 year and 2 years was 7% and 13%. Causes of death were refractory GVHD (n=1) and infection (n=1). Treatment response was evaluated in 14 pts: complete remission was achieved in 13 pts (93%), MRD negativity after SCT was observed in 11 pts. With median follow-up from SCT 27 months (range 3-33), 80% of all pts (12/15) were alive (11 in remission, 1 with relapse), 3 pts died (2 from NRM, 1 from relapse), 3 relapses (21%; 3/14) occurred.
Conclusion: FC-RIC protocol represents a promising approach to the treatment of poor-risk CLL with high reponse rate (93%);

progression-free survival and overall survival at 2 years from SCT were 67% and 80%, respectively.

**P1087**

**Survival following allogeneic haematopoietic cell transplantation after non-myeloablative conditioning depends on day 100 PET scan in patients with lymphoma**

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Background: PET scan is increasingly used in the follow-up of lymphoma patients (pts). Whereas several studies addressed the question of the impact of PET positivity after autologous transplantation on transplantation outcomes, very few have been performed after allogeneic hematopoietic cell transplantation (Allo-HCT). This is the aim of the current retrospective study.
Methods: We analyzed data from 50 lymphoma pts who underwent an allo-HCT after non-myeloablative conditioning. The diagnoses were Hodgkin's lymphoma (n=8) and non-Hodgkin's lymphoma (n=42). PET scans were scheduled on days 100,180 and 365 and then yearly for a total of 5 years.
Results: Day 100 PET scans were not performed in 5 patients. Among the remaining 45 patients, 20 (44.4%) presented hypermetabolic lesions, including 9 patients (20%) with lesions evocative of lymphoma.1-year overall survival (OS) (Figure 1) was lower in patients with typical lymphoma lesions than for those whose PET scan was negative or positive for infectious/ inflammatory reasons (44% *vs* 85%, p=0.0013).
Figure 1 : OS according to 1st PET scan on day 100 (0=complete response, 1=inflammatory/infectious, 2=probably inflammatory/ infectious, 3=residual disease, 4=progression or new lymphomatous lesion, 5=suspicion of other neoplasia).
During follow-up, twenty pts (44.4%) never presented hypermetabolic lesions after transplant and 25 (55.6%) had at least one abnormal PET scan.
Among the 25 patients, only 9 (20%) had probable/proven lymphoma: 3 residual diseases, 5 relapses and 1 non-biopsy proven progression. Two others pts (4.4%) presented another neoplasia (1 lung cancer and 1 lung PTLD).
The 14 remaining pts (31.1%) had suspicious lesions at one of the follow-up PET scans, but none of these proved to be a relapse. Biopsies were performed in 6 of these cases, including 2 lymph node (1 normal and 1 lymphoid hyperplasia), 2 lung (1 normal and 1 aspergillosis) and 2 gastro-intestinal (1 normal and 1 Graft-versus-Host disease) biopsies. For 6 pts, imaging studies were normal or demonstrated infectious or inflammatory disorders. The last 2 pts were thought to relapse based on both PET and CT scans, refused biopsies, but then their lesions regressed spontaneously.
Conclusion: A positive PET scan on day 100 post-transplant is predictive of poorer OS. However, there is a noteworthy incidence of false-positive PET scans after non-myeloablative allo-HCT. We therefore recommend that every suspicious lesion should be explored by CT scan and/or biopsy.

**P1088**

**Tandem autograft/reduced-intensity allograft compared with autograft for relapsed/refractory hodgkin's lymphoma not in complete remission after salvage chemotherapy**

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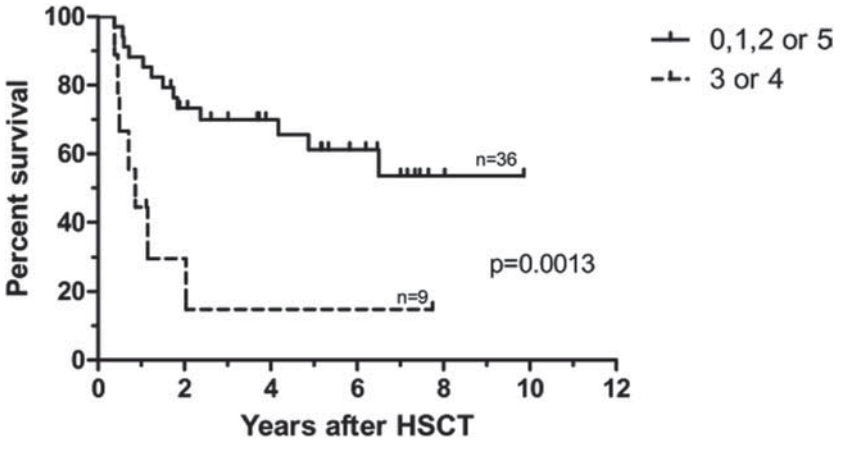
Hodgkin's lymphoma (HL) patients often have poor prognosis when primary refractory or relapsed after first line treatment; salvage chemotherapy followed by high-doses chemotherapy and stem cells reinfusion (auto-SCT) represents the standard therapy. This treatment cures 15-30% of primary refractory and 50% of relapsed patients. New therapeutic strategies are necessary for those patients. Autologous stem cell transplant (autoSCT) followed by reduced intensity allogeneic SCT (tandem autoSCT-alloRIC) is a feasible approach exploiting high dose chemotherapy effect and graft *vs.* HL effect. In this retrospective study we compared the outcome of two groups of patients, not in complete remission (CR) after one or multiple salvage chemotherapy lines which performed a single autoSCT (n=34) or, in case of availability of an allogeneic donor, a tandem autoSCT-alloRIC (n=35). Patients groups were balanced: 70% were primary refractory and 30% relapses in both groups; 79% of autoSCT group and 69% of tandem group received one salvage chemotherapy line; 56% of autoSCT group and 40% of tandem group where in partial remission (RP) whereas the remaining patients in both stable (SD) or progressive (PD) disease before transplant. In comparing the two groups there wasn't difference in disease response after transplant procedures (30 days). Univariate analysis showed a statistically significant advantage in progression free survival (PFS) for the tandem *vs.* the autoSCT group (11.5 *vs.* 6.6 months, p=0.013) and an advantage in overall survival (OS) not reaching statistical significance (Nr *vs.* 35.3 months, p=0.165). In multivariate analysis patients treated with autoSCT have a progression risk 3 times higher compared to patients treated with tandem (HR 3.11 (1.67; 5.85), p<0.001). After autoSCT 24/34 patients progressed (70%) whereas 18/35 patients after tandem (51%). After a median follow up of 52 months, 20/34 patients (59%) of autoSCT group and 23/35 patients (66%) of the tandem group were alive, of those 16 (80%) and 19 (83%) respectively



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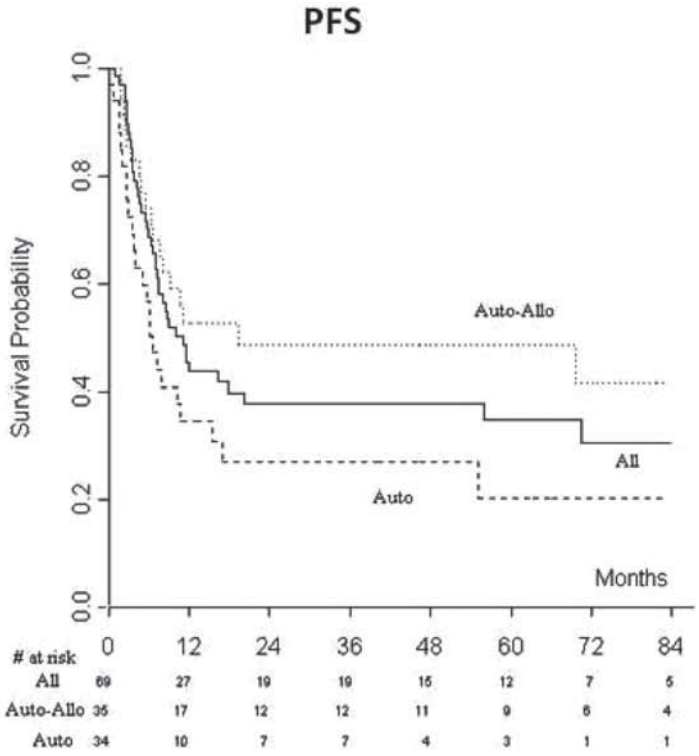


in CR; 14 patients (41%) of the autoSCT group and 12 patients (35%) of the tandem group died. In both groups 10 patients died of PD while 4 (11%) of the autoSCT and 2 (6%) of the tandem group for toxic events. Although we can't draw definitive conclusions about the tandem efficacy for the retrospective nature of the study, our results suggest that tandem auto-SCT-alloRIC ameliorated the outcome of those patients.

**P1089**

**High-dose rituximab in the conditioning regimen before allogeneic stem cell transplantation for relapsed lymphomas: protective role of the antibody on GvHD incidence**

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Allogeneic stem cell transplantation (alloSCT) with a reduced-intensity conditioning (RIC) is an effective salvage therapy for relapsed lymphomas. The addition of Rituximab (R) in the conditioning could enhance the anti-lymphoma effect. The high-dose R before alloSCT was studied by MD Anderson group in indolent (LG), but not in aggressive (HG) lymphomas. We designed a prospective, multicenter, phase II study.
Objective: Primary end-point was 1-year progression-free survival; secondary endpoints were non-relapse mortality, incidence of acute and chronic graft-versus host disease (GVHD).
Methods: Treatment plan consisted of high-dose R (500 mg/ms), thiotepa (12 mg/kg), fludarabine (60 mg/kg) and cyclophosphamide (60 mg/kg). GVHD prophylaxis included cyclosporine and short course methotrexate; ATG (7 mg/kg) was added for pts allografted from one antigen mismatched sibling or unrelated donors. Fifty-four pts are now evaluable. Histopathological subtypes included 25 HG (n=18 diffuse large B-cell lymphomas, n=7 mantle cell lymphomas) and 29 LG (n=15 follicular lymphomas, n=14 chronic lymphocytic leukemia). Pts were allografted from related siblings (SIB) (n= 34 matched, n=1 one mismatched) or unrelated donors (UD) (n=15 matched, n=4 mismatched). Ten of 25 pts (40%) with HG and 11 of 29 pts (38%) with LG were in complete remission at time of allo-SCT.
Results: At median follow-up of 12 months (range, 5-44 months), 41 pts are alive and 13 died: 6 for non-relapse mortality (NRM), and 7 for disease. The cumulative incidence (CI) of NRM was 12% at 1 year [9% versus 16% for alloSCT from SIB and MUD (p=0.3)]. In total only 13 of 54 patients had acute GVHD (n=10 grade II, n=3 grade III) with an estimated CI of 23% at 100 days. Interestingly, acute GVHD was inferior in pts allografted from MUD donor where the ATG and R combination was used (11% versus 29% from MUD and SIB donors, respectively). The main infectious complications were: n=4 (8%) sepsis, n=10 (19%) pneumonia, n=3 (6%) viral (no CMV), n=2 (4%) fungal. The CI of relapse at 3 years was 38% and 51% in LG and HG lymphomas, respectively. The 2-years OS and PFS were 79% and 53% for LG and 61% and 33% for HG lymphomas, respectively.
Conclusions: We observed: 1) reduced incidence of acute GVHD especially in pts receiving a combination of ATG and R; 2) the outcome of pts affected by HG was not improved by the administration of R.

**P1090**

**Reduced-intensity alemtuzumab-based allogeneic transplant outcomes in elderly patients with lymphoproliferative disorders are affected by Sorror co-morbidity index score and disease status at transplant**

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Reduced intensity conditioned (RIC) allogeneic transplantation represent a potentially curative therapy in older patients with lymphomas. Given the increased incidence of lymphomas in these patients alemtuzumab RIC allografts are increasingly used but to date the tolerability and activity has not been studied.

Aim of the Study: To assess transplant outcomes in elderly patients with lymphomas(overall survival OS, disease free survival DFS, 1 yr Transplant related mortality TRM, Graft versus Host disease GvHD) and correlate transplant outcomes with the Sorror comorbidity index. As part of the quality of life assessment we also looked into the number of inpatient days in the first 100 days post transplant and correlated them with patients' pre-transplant characteristics.
Materials and Methods: 47 patients(26 male and 21 female) above the age of 60 with lymphomas from 2 transplant centres were included in the study. The median follow up length was 26 months (range 1-96 months) and the median age 64 years (range 60-70). 17 patients had follicular lymphoma, 12 had CLL, 2 had Waldenstroms, 5 with Hodgkins, 3 diffuse large B cell lymphoma and 3 splenic marginal zone lymphoma. 31 patients received a peripheral stem cell graft from a fully matched unrelated donor and 16 from a sibling donor.26 patients were transplanted in CR and 21 in PR.
18 received conditioning with Fludarabine/Melphalan/alemtuzumab 14 patients with BEAM/alemtuzumab,9 with Fludarabine/BEAM/alemtuzumab, 6 with Fludarabine/cyclophosphamide/Alemtuzumab.Sorror comorbidity scoring was performed in all patients. 12 patients had a comorbidity index of 3 and above.
Results: All patients engrafted. The two years OS was 56% .TRM in the 1st year was 61% for those with a Sorror comorbidity index of ≥3 but only 12% for those with a score <3. The relapse rate was 30% in 2 years. Two factors were associated with decreased OS and reduced DFS: a sorror comorbidity index of ≥3 and partial remission pre transplant. CMV status,stem cell dose and graft source were not significant on OS and DFS. A Sorror modified comorbidity index for HCT of 3 and above was associated with increased inpatient stay (P:0.001)
Conclusion: These data demonstrate that alemtuzumab RIC allografts can be delivered safely in selected patients above the age of 60. Sorror comorbidity scoring is a simple method which can identify older patients with inferior outcomes and should form the basis for pre-transplant assessment in this patient population.

**P1091**
**Rh D allo-immunisation is uncommon following myeloablative and reduced-intensity conditioning allogeneic haematopoietic stem cell transplantation**
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Background: Rh D incompatibility between donor and recipient occurs commonly in allogeneic HSCT and some recipients may be alloimmunized to Rh D prior to transplantation. With increasing use of reduced intensity conditioning (RIC) regimens, the safety of antigen-incompatible transplants remains a concern.
Study Design and Methods: All patients undergoing allogeneic HSCT between 2000 and 2010 at a large Canadian teaching hospital were reviewed for Rh D incompatibility. The primary endpoint was the development of Rh D antibodies.
Results: A total of 419 patients underwent allogeneic transplantation between 2000 and 2010. 79 patients (19 %) were mismatched for the Rh D antigen. Of 16 patients who underwent RIC transplant, none showed evidence of anti-D alloimmunization post-transplant. None of the patients who underwent myeloablative conditioning developed new Rh D antibodies after transplant. One Rh D negative patient with evidence of anti-D before transplantation underwent myeoloablative conditioning and received an Rh D-positive donor graft. The patient experienced significant hemolysis that resolved within 30 days of transplantation, despite persistence of anti-D at last follow-up, 16 months after transplantation.
Conclusion: Rh D alloimmunization is rare following myeloablative and RIC allogeneic HSCT. One patient had persistence of anti-D after transplant with no long-term clinical sequelae.

## Solid Tumours

**P1092**
**Effective preclinical activity of cytokine induced killer cells against autologous metastatic melanoma including cells with tumour initiating features**
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Adoptive immunotherapy strategies hold great promises for treatment of metastatic melanoma, incurable with conventional chemotherapies.
Crucial issues for their clinical translation are the availability of "clinically adequate" numbers of immune effectors, the effective killing against autologous tumors and ideally the efficacy against tumor initiating cells (TIC).
We investigated the preclinical efficacy of Cytokine-Induced Killer (CIK) cells against autologous metastatic melanoma cells including melanoma-TIC (mTIC). CIK cells are a subset of *ex-vivo* expanded T lymphocytes, with mixed T-NK phenotype and endowed with MHC-unrestricted antitumor activity. We

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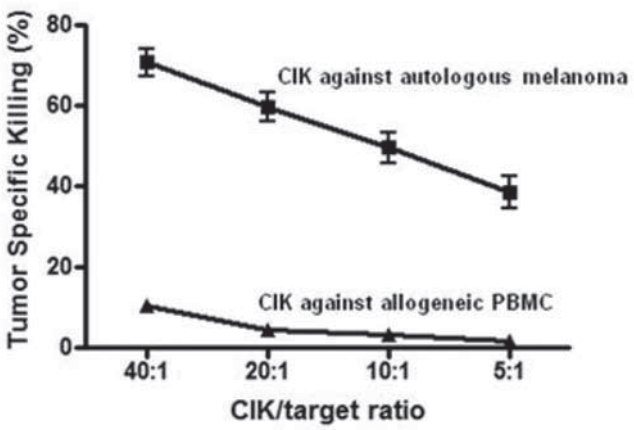


**Fig.1 Tumor Killing of CIK cells against autologous Melanoma**

successfully *ex-vivo* expanded CIK cells from 10 patients with metastatic melanoma. CIKs were generated from PBMC with the timed addition of IFN-gamma; Ab anti-CD3 and IL2. Expanded CIKs included a median of 41% CD3+CD56+ cells (range, 21-80). The median *ex-vivo* expansion for the CD3+CD56+ fraction was 318 fold (109-1870). CIK cells efficiently killed *in vitro* autologous metastatic melanoma cells with an average specific killing of 71%, 60%, 49% and 38% at 40:1, 20:1, 10:1 and 5:1 effector/target ratio respectively (n=23) (Fig. 1). Autologous tumor cell lines, used as targets, were generated from all our patients and confirmed to express membrane molecules (MIC A/B, ULBPs) recognized by CIK cells.
We investigated the ability of CIK cells to kill mTIC. To identify mTIC, we transduced bulk melanoma cells with a lentiviral vector encoding for the enhanced Green Fluorescent Protein (eGFP) regulated by the human OCT4 promoter. The underlying idea is that mTIC can be visualized based on their exclusive ability, proper of both normal and cancer stem cells, to activate the OCT4 promoter and consequently express eGFP. The average presence of eGFP+mTIC within bulk melanoma cells was 12.4 ± 6.7%. CIK cells efficiently killed autologous, eGFP+ sorted, mTIC; the average killing was 68%, 55%, 43% and 33% at 40:1, 20:1, 10:1 and 5:1 effector/target ratio respectively (n=5), results overlaid those observed against the counterpart of eGFP- tumor cells. We reported for the first time the activity of CIK cells against autologous metastatic melanoma. The easy *ex-vivo* expansion and the MHC-unrestricted killing of CIK cells may favour their clinical translation. The efficacy against putative mTIC can potentially address relevant clinical issues, targeting tumor cells responsible for chemo-resistance and disease relapse.

**P1093**
**Matched sibling haematopoietic stem cell transplantation for treatment of recurrent medulloblastoma with bone marrow disease in children; favourable response of minimal residual disease detected by low-density arrays**
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We report our to date experience in one child with recurrent metastatic anaplastic/large cell (A/LC) medulloblastoma. A 2.5 year old male presented in December 2008 with disseminated A/LC medulloblastoma. He received multi-agent induction chemotherapy January-April 2009, with radiographic MRI response, followed by consolidation chemotherapy through June 2009, when brain and spine MRI demonstrated widespread leptomeningeal recurrence. He received 3600cGY craniospinal irradiation with 5400cGy posterior fossa boost July-August 2009. Within 4 months he began complaining of back and leg pains; brain and spine MRI were negative for CNS tumor; pelvic and spine MRI demonstrated widespread bone metastases. Iliac crest biopsy was positive for medulloblastoma. He received salvage chemotherapy April-August 2010 with achievement of incomplete remission with radiological evidence of persistence bone and bone marrow disease and no evidence of residual CNS disease. Attempt at harvesting autologous hematopoietic progenitor cells was unsuccessful.
His 9 year old brother was histocompatible and the patient undergone conditioning chemotherapy with thiotepa, melphalan, etoposide and fludarabine followed by Hematopoietic Stem Cell Stem Transplantation (HSCT) from his brother's bone marrow in September 2010.
Pre-HSCT evaluation of his bone marrow with Low Density Arrays (LDA) showed evidence of minimal residual disease (MRD) which resolved on re-evaluation post-HSCT.
Radiological evidence of residual disease resolved approximately 6 month after BMT. He tolerated treatment uneventfully, and was discharged from hospital on day +47. He subsequently developed mild, transient skin chronic graft-versus-host disease (GvHD).
On Day+304 post-HSCT patient had asymptomatic radiological evidence of local recurrence in the brain posterior fossa of the brain with no evidence of extra-cranial metastases/recurrence. Subsequently, he received intra- omaya chemotherapy with stabilization of his recurrent disease and is currently receiving intra-omaya radiolabeled monoclonal antibodies with activity against GD2 protein.
Currently patient displays full marrow reconstitution, no GvHD, no bone pain and an excellent quality of life with a Lansky PS of 80.
Allogeneic HSCT may be a consideration for high risk recurrent medulloblastoma patients with bone and bone marrow disease. LDA can be utilized as a tool for assessment of metastatic medulloblastoma MRD in bone marrow.

**P1094**
**Haploidentical SCT for neuroblastoma. A subtle balance between "mini" conditionings and "mega" grafts**
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The few reports analyzing outcomes of haploSCT in neuroblastoma (NB) that are available in the literature are heterogeneous because they include patients with refractory disease as well as patients who relapsed, patients in complete remission as well as patients in progressive disease (PD), pts transplanted after different conditioning regimens and with grafts obtained after relatively hetrogenous procedures of T-cell depletion. All of these factors preclude the possibility of drawing a reliable conclusion on the role of haploSCT in NB.
We evaluated the feasibility and efficacy of using reduced-intensity conditioning (RIC) and transplantation of haploidentical PBSC to treat high-risk (initially stage IV refractory/relapsing) NB. 5 patients were enrolled, all after autoSCT: 4 after relapse and 1 with refractory disease. Median time from diagnosis to haploSCT was 28 months (13-77). The RIC included fludarabine 180 mg/m$^2$, busulfan 8 mg/kg and TBI 2Gy. CD3/CD19 cell-depleted maternal stem cells were infused on day 0 and no immunosuppression was given. 3 received post transplant donor NK cell infusions. RIC was very well tolerated with no TRM. 3 pts engrafted and 2 experienced graft failure (one patient rejected the graft and was rescued with autologous backup and one experienced "nonengraftment" and was retransplanted with stem cell from his father. One primary grade I acute GvHD was observed and successfully treated. One patient died of PD 6 months after transplantation (he was transplanted when in PD and received post SCT immunosuppressive treatment because of high dose of CD3+ cells in the graft). 4 patients are alive: 2 in PD (1 who was rescued with autologous cells), 2 are alive and well with short follow-up (1 after 2nd haploSCT). The first aim of our study was to analyze faesability of haploSCT in NB. While it is difficult to draw definitive conclusions based on this low number of patients a speculative explanation for the poor result is the imbalance between transplantation-related factors, such as the type of conditioning, the lack of serotherapy, and the number of CD34+ and CD3+ cells, which influence on successful engraftment. To improve outcome of haplo for high-risk NB we shall reconsider: use of antithymocyte globulin, giving a graft with higher number of CD34+ cells and reassess amount of T-cell depletion. The last message of our study is that haploSCT should not be considered an option if stable remission is not achieved before transplantation.

**[P1094]** Table1. Patient and graft characteristics and outcome

| Pt | Sex Age | 1st line treatment | Relapse | Post relapse treatment | Status at haploSCT | Graft CD34+ | Graft CD3+ $10^6$/kg | NK | Days to ANC > 0.5 $10^9$/L | Days to PLT> 50 $10^9$/L | Chimerism D+30 | DNKI D+30 $10^6$/kg | DNKI D+60 $10^6$/kg | Follow up post haploSCT Months | Follow up post haploSCT Status |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M/4 | COJEC, Surgery Bu-Mel, Rx, Retinoic ac. | Yes | TOTEM | Relapse post $CR_2$ | 10 | 0.76 | 37.5 | 13 | 13 | full | 10 | 10 | 6 | DCD from PD |
| 2 | M/1.5 | COJEC, TVD Surgery, MITOP Bu-Mel | non | - | Refractor $PR_1$ | 19 | 0.01 | 30 | 14 | 10 | graft rejection autoSCT at D+38 | | | 4 | Alive PD |
| 3 | M/3.5 | COJEC, Surgery Bu-Mel, Rx, Retinoic ac. $GD_2$ | Yes | CADO | $VGPR_2$ | 18 | 0.045 | 96.8 | 18 | 9 | full | 10 | 10 | 3 | Alive PD |
| 4 | F/6 | COJEC, Surgery Bu-Mel, Rx, Retinoic ac. | Yes | TVD | $PR_2$ | 7 | 0.025 | 110 | "nonengraftment" 2nd haploSCT at D+25 | | | | | 2 | Alive $VGPR_2$- |
| 5 | M/5.5 | COJEC, TVD, Surgery, Bu-Mel, Rx, Retinoic ac. | Yes | TOTEM | $PR_2$ | 7.2 | 0.029 | 25.2 | NE | NE | NE | NE | NE | 1 | Alive well |

COJEC, cyclophosphamide, vincristine, etoposide, carboplatin, cisplatin; TOTEM, temozolomide, topotecan; TVD, temozolomide, vincristine, doxorubicne; Rx, radiotherapy; Bu-Mel, busulfan, melphalan; DNKI, donor NK infusion; NE no evaluable ongoing SCT procedure

**P1095**

**Unmanipulated HLA haploidentical haematopoietic stem cell transplantation for refractory/relapsed paediatric solid tumour**

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Background: Despite intensive multi-modal therapies, prognosis of refractory/relapsed pediatric solid tumor is dismal. Therefore new break-through approaches should be expected in order to improve survival. We hypothesized that unmanipulated haploidentical hematopoietc stem cell transplantation (UH-SCT) could be a novel platform of immunotherapy based on powerful graft-versus-tumor effect induced by alloreactive T and NK cells. However lethal graft-versus-host disease (GVHD) and graft failure should be overcome to perform UH-SCT safely.
Objectives and Methods: This study presents a series of transplant experiments aiming to evaluate the efficacy and feasibility of UH-SCT for refractory or relapsed pediatric solid tumors. Eight cases with refractory/relapsed pediatric solid tumors were enrolled in this study from July 2007 to December 2011. One patient had a second transplantation due to tumor progression at 1 year after first transplantation. Among 8 patients, there are 3 cases of relapsed neuroblastoma, 1 case of relapsed mesenchymal chondrosarcoma, 2 cases of relapsed Ewing sarcoma, 1 case of refractory alveolar soft part sarcoma with multiple lung metastases, and 1 case of refractory primitive neuroectodermal tumor. Conditioning regimens were fludarabine+Melphalan+rabbit-anti-thymocyte-globulin.
(ATG) and GVHD prophylaxis consisted of the combination of tacrolimus, methotrexate, and predonisolone.
Results: All patients achieved primary engraftment however secondary graft rejection was observed in one patient. HLA disparities were 3/8 in 2, 4/8 in 6, and 2/8 with KIR mismatched in 1. Five patients received peripheral blood stem cells and 4 patients received bone marrow stem cells. Incidence of acute GVHD was 5/8 cases (grade II-IV: 2 , II-IV: 1), chronic GVHD was observed in all 6 evaluable patients. Four cases received donor lymphocyte infusion (DLI) due to tumor progression. Transplant-related mortality (TRM) was not observed. Five cases had tumor progression, and 3 of 5 cases died, the other 2 cases are alive with additional treatment. Three cases are alive and well for 3, 16 and 17 months after transplantation without progression. With the median follow up of 16 (3-43) months, the two-year probability of overall survival was 71.4% in this study.
Conclusion: These results indicated the feasibility and the efficacy of UH-SCT for relapsed or refractory pediatric solid tumors.

**P1096**

**Autologous heterotopic transplant of peripheral blood complementary of neoadjuvant chemotherapy in solid tumours**

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Background: Autologous bone marrow transplantation was proposed as a procedure of cancer immunotherapy based in the graft-versus host effect. In that model, graft/receptors were not conditioned to generate immunity responses, and frequently, the patients were immune-suppressed. Over the past few years, our team has developed an Autologous Heterotopic Transplant Of Peripheral Blood (AHTOPB), a procedure including a strong conditioning of graft/receptors to elicit antitumoral responses.
Methods: The state of art in AHTOPB configuration is reported here for the first time:
1. Week 1 to 4: Patient conditioning with drugs that switch the malignancy-induced-tumor-progressive conditioning of the systemic microenvironment (neoangiogenesis/immune-tolerance).
2. Week 5 to 8: Transplant *ex-vivo* conditioning to generate a subcutaneous/intramuscular site where injections of specific antitumoral immunization do not elicit tolerogenic responses.
3. Finally, thermostable autologous plasma fraction, previously reported as a fraction containing antigens released from tumors and conserved inside thermostable stress shock proteins, was injected in the prepared immunogenic site.
The evidence in independent reports for each step of this configuration is revisited.
Patients: From clinical trials performed to test AHTOPB and previously published, control and treated patients, accomplishing the inclusion and exclusion criteria, were selected and the assessments statistically analyzed.
Inclusion criteria: Primary solid tumors: Pancreas, Colorectal, NSCLC, Ovary, Prostate and Breast. Non-resectable, M1, treated with standard neoadjuvant chemotherapy. RECIST tumor growth in progression. PS ≤ 2.
Exclusion criteria: Treated co-morbidity; Brain metastasis. Surgery not performed. Less than 3 month of treatment.
For each primary localization, patients were included in 2 groups G1, treated with the standard chemotherapy and G2, treated with Chemotherapy and AHTOPB.
Assessment: Monthly, toxicities ≥ 3 (CTCAE), No of cases in Stable Disease according RECIST and Overall Survival.

[P1096]

Table

| | Colorectal | | | | Prostate | | | | Breast | | | | Ovary | | | | NSCLC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G1 | 18 | 6 | 2 | 20.4 ± 4.2 | 14 | 2 | 3 | 51.4 ± 6.6 | 24 | 3 | 5 | 26.8 ± 4.1 | 8 | 1 | 0 | 50.0 ± 6.2 | 14 | 4 | 1 | 9.6 ± 1.2 |
| G2 | 20 | 7 | 8 | 32.7 ± 3.7 | 14 | 1 | 10 | 84.2 ±4.8 | 20 | 2 | 14 | 34.2 ± 5.0 | 10 | 1 | 4 | 61.2 ± 4.9 | 15 | 5 | 7 | 16.1 ± 1.1 |
| | n | TX ≥ 3 | StD | OS mean ± SD | n | TX ≥ 3 | StD | OS mean ± SD | n | TX ≥ 3 | StD | OS mean ± SD | n | TX ≥ 3 | StD | OS mean ± SD | n | TX ≥ 3 | StD | OS mean ± SD |

n: Number of cases StD: Stable Disease (n) OS: Overall Survival (months) TX: Toxicities CTCAE ( n) ☐ p<0.05

| | VEGF | | ANGIOSTATIN | | T-Regulatory cells | | Activated Dendritic Cells | | Lymphocyte Proliferation Assay | |
|---|---|---|---|---|---|---|---|---|---|---|
| DB | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| TS mean ± SD | 105 ± 9.8 | 60 ± 7.2 | 96 ± 6.1 | 210 ± 2.6 | 112 ± 8.8 | 48 ± 1.6 | 32 ± 2.2 | 148 ± 7.8 | 98 ± 8.0 | 305 ± 13.1 |
| BP mean ± SD | 118 ± 10.8 | 74 ± 6.7 | 76 ± 3.2 | 178 ± 8.4 | 132 ± 9.2 | 52 ± 2.5 | 30 ± 2.4 | 126 ± 6.9 | 102 ± 7.6 | 201 ± 11.0 |
| | 78 | 79 | 78 | 79 | 78 | 79 | 78 | 79 | 78 | 79 |
| | G1 | G2 | G1 | G2 | G1 | G2 | G1 | G2 | G1 | G2 |

DB: Diagnosis biopsy TS: Tumor surgery BP: Blood plasma. Results expressed as % of mean value at DB time ☐ p< 0.05

At time of diagnosis and post-treatment surgery, Lymphocyte Proliferation Assay (Thymidine-H3), Angiostatin and VEGF, T-Reg (ELISA) and Activated dendritic cells (Immunohistochemistry).
Results: Shown in Table.
Conclusions: AHTOPB last configuration confirms safe antitumoral effect, improving the neoadjuvant activity of chemotherapy in different advanced solid tumors, stimulating future developments of this procedure.

**P1097**
**Autologous stem cell transplantation for high-risk Ewing's sarcoma: a single-centre review**
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Introduction and Objective: The prognosis for many pediatric and young adult patients (pts) with Ewing Sarcoma that have metastasized at the time of diagnosis or relapsed after therapy remains very poor. High dose chemotherapy with autologous stem cell transplantation (ASCT) has been employed as consolidation therapy for pts with a variety of high-risk solid tumors. Herein, we review a cohort of pts with diagnosis of Ewing Sarcoma that underwent ASCT.
Methods: The data were collected from individual pt records registered in our BMT Unit as autologous transplant, from January 2001 to September 2011.
Results: Twenty-three pts underwent to ASCT, 16 male and 7 female, with a medium age of 18 years. Histologic diagnosis was Ewing sarcoma, with following distribution: axial skeleton (19pts) and extremities (4pts). At time of diagnosis, 17 tumors were metastatic (74% pts), with lung (15 cases) and bone metastasis (3 cases). Initial treatment consisted of chemotherapy, surgical resection (11pts) and lung metastasectomy (2pts). Pts were submitted to ASCT, with a median of 10 months after diagnosis. Disease status at the time of ASCT was: partial response (8pts), complete response (14pts), refractory disease (1pt). The conditioning regimen was busulfan and melphalan. Stem cell source was peripheral blood stem cells in 21 pts and autologous bone marrow in 2 pts. A medium of $6.73x10^6$ nucleated cells per kg was infused. All pts achieved engraftment. The median time to neutrophil engraftment was 12 days (range: 10-16 days), and to platelet engraftment was 11 days (range: 7-34 days). Two pts failed to achieve platelet engraftment. Transplant related complications were: hepatic veno-occlusive disease (VOD) (2pts), febrile neutropenia (21pts), bacteremia (4pts), candidemia (1pt), clostridium difficile colitis (1pt), pneumonia (1pt) and grade IV mucositis (15pts). One death occurred on day+20, due to VOD complicated by multi-organ failure. One secondary acute myeloid leukemia was diagnosed 12 months after ASCT. Ten pts died due to progressive disease, which occurred at a median of 9 months after ASCT. Thirteen pts are alive with a median follow-up of 13 months after ASCT and 10 of them remain in complete remission. Conclusion: In this cohort of pts, conditioning regimen had acceptable toxicity, with only one death conditioning regimen-related. Metastatic disease was registered in 74% pts, which may contribute to poor prognosis, although, 43% of pts remain disease free.

**P1098**
**Autologous stem cell transplantation in Ewing's sarcoma: the experience of Rambam medical centre and Tel Aviv medical centre**
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Introduction: In contrast with the dramatic improvement in survival for localized Ewing sarcoma (ES) during the last 30 years, with 5-year overall survival (OS) of 70%, the prognosis of patients (pts) with multifocal primary disease or with early

relapse remains poor. However, pts with primary pulmonary metastases treated either by conventional therapy or by autologous stem cell transplantation (ASCT) fared better than pts with other metastatic sites. The experience of two centers, Rambam medical center and Tel Aviv medical center is described herein. Patients: Sixteen consecutive pts, 11 boys and 5 girls, underwent ASCT between the years 1999-2011. Median follow-up was 36 months (range: 7.9-152 months). Median age was 14 years (range: 2-21 years). Primary tumor site was in the pelvic area in 7 pts, chest in 3 pts, tibia in 2 pts, and spine, axila, humerus, and femor in 1 pt each. Degree of tumor necrosis after surgery was >90% in 9 pts, <90% in 6 pts, and no surgery in 1 pt. Thirteen pts were in 1st complete remission (CR) before transplantation, 2 were in 2nd CR, and 1 pt was in progressive disease. Indications for transplantation were pulmonary metastasis in 7 pts, relapse in 2 pts, poor response to chemotherapy in 5 pts, bone and BM spread in 1 pt, and pelvic mass >200 ml in 1 pt. Conditioning regimen included busulfan and melfalan (Bu/Mel) in 12 pts, and other regimens in 4 pts. Pharmacokinetic analysis of Bu plasma concentrations was preformed after the second dose of Bu. When the area under the curve (AUC) was found to be outside the therapeutic window (850–1,200 M×min), the drug dose was adjusted at the fifth dose. Results: All 16 patients engrafted. Median time for neutrophil engraftment was 11 days (range: 10-17 days). No patient developed veno- occlusive disease (VOD). Eleven patients are alive. Causes of death were relapse post SCT in 4 pts and death due to CMV infection post SCT in 1 pt. Six years overall survival (OS) was 56%.Conditioning regimen with Bu/Mel achieved statistically significant better OS compared to other regimens (P=0.0362). Eight years disease free survival was 41%. Six patients relapsed after SCT, only 2 survived. Conclusion: Six years OS of 56% was documented in a cohort of 16 consecutive ES pts after ASCT. Statistically significant better OS was documented in patients receiving BU/Mel as conditioning regimen. Adjusting BU dose is important and may account for the lack of VOD demonstrated in our pts.

**P1099**

**Multi-cycle high-dose chemotherapy with ti-ce regimen for relapsed/refractory patients with germ cell tumours: the Romagna cancer institute experience**

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Background: Recently developed at the Memorial Sloan Kettering Cancer Center (MSKCC) (Kondagunta *et al* – JCO 2005 and Feldman *et al* – JCO 2010) for patients with relapsed germ cell tumors (GCT), the TI-CE regimen is a new multi-cycle high-dose chemotherapy (HDCT) regimen comprising a mobilizing phase with paclitaxel and ifosfamide (TI) followed by 3 HDCT courses with carboplatin and etoposide (CE) and hematopoietic progenitor cell support. We present preliminary results obtained at the Romagna Cancer Institute (I.R.S.T.) using this regimen at either first or further relapse.
Methods: From August 2009 to December 2011, 22 patients with relapsed/refractory GCT were referred to our Institute for salvage HDCT. Median age was 34 years. HDCT as first-relapse salvage therapy was scheduled for 16 patients and as second-relapse treatment for the remaining 6. Biosimilar filgrastim (Zarzio®) was used in the HDCT phase for all patients after each peripheral blood progenitor cell (PBPC) reinfusion.
Results: Two of 22 patients were treated with TI but did not receive CE: the first had rapidly progressive disease (PD) with a decline in performance status, while the second refused HDCT. Of the remaining 20 patients, 16 have now completed the TI-CE regimen and 4 are still undergoing treatment. A total of 41 CE cycles were administered to the 16 evaluable patients, of whom 12 (75%) received all three planned CE cycles and 4 received 1 or 2 CE cycles due to either early progressive disease (n=2) or poor PBPC collection (n=2). There were no treatment-related deaths. The median number of days from the start of CE until recovery of neutrophils to 1,000/mm$^3$ was 15. Ten (62.5%) of the 16 evaluable patients achieved a complete remission (CR) (4 clinical CR, 5 pathological CR, 1 surgical CR), 2 had marker-negative partial remissions lasting 3 and 20+ months, 1 showed stable disease lasting 3+ months and 3 progressed. Thirteen (82.5%) of the 16 patients are currently alive progression-free after a median follow-up of 12 months.
Conclusions: Our experience would seem to indicate that the TI-CE regimen is safe and active, with a response rate and a recovery time of neutrophils after CE similar to that reported in the MSKCC experience. A large international phase III randomized trial (TIGER study) has been recently planned to compare the TI-CE regimen with standard chemotherapy as first salvage chemotherapy.

**P1100**

**Results of high-dose chemotherapy with autologous haematopoietic stem cell transplantation in the treatment of paediatric brain tumours**

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Aim: Central nervous system (CNS) tumors are the second most common pediatric malignancies with an about 30% 5-year overall survival rate in high-risk group. The aim of this study was to assess the effectiveness of single or tandem high-dose chemotherapy (HDCT) with autologous hematopoietic stem-cell transplantation (auto-HSCT) in this patient group.
Methods: From October 2006 to September 2011, 11 pediatric patients with high-risk medulloblastoma (N=6), supratentorial PNET (N=3), and germinoma (N=2) received HDCT with auto-HSCT after induction chemotherapy and surgical treatment. At the moment of HDCT 3 patients were in complete remission (CR), 4 patients were in partial remission (PR) and 4 patients had stable disease (SD). Patients with germinoma received single auto-HSCT, patients with medulloblastoma or supratentorial PNET received tandem auto-HSCT. The conditioning regimen in the case of single auto-HDCT consisted of cisplatin, etoposide, and ifosfamide. In tandem HDCT, a carboplatin and etoposide regimen ± thiotepa was followed by a thiotepa and cyclophosphamide regimen. Bone marrow (n=4), peripheral blood stem cells (n=4) or both (n=3) were used for stem cell sources. The mean transplanted CD34+ cell dose was 5.27x10$^6$/kg (range, 1.0-8.9x10$^6$/kg).
Results: The median follow-up for living patients is 10 months (range, 3-60). All patients with SD at the moment of transplantation died due to progression. Three of 7 patients with CR or PR relapsed 1-9 months after HDCT, 2 of them were in 2nd remission prior to HDCT, the other 4 patients are currently in CR. The conditioning regimens used were characterized by reasonable toxicity (5 patients had grade 3-4 mucositis, 6 patients had grade 3-4 infection). The median time to engraftment was 16 (range 12-30) and 17 (range 13-86) days after the first and second auto-HSCT respectively.
Conclusions: HDCT with auto-SCT in pediatric patients with high-risk CNS tumors is characterized by acceptable toxicity and may be a feasible option for patients in CR or PR after induction chemotherapy. It is ineffective as a salvage therapy in refractory patients.

**P1101**

**Cytomegalovirus reactivation is associated with a lower incidence of relapse after allogeneic stem cell transplantation for chronic myelogenous leukaemia**

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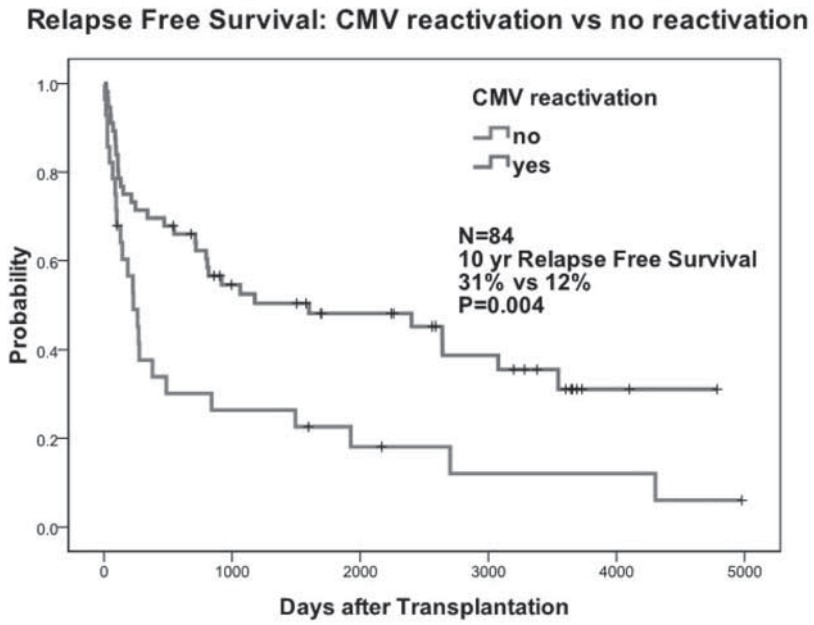
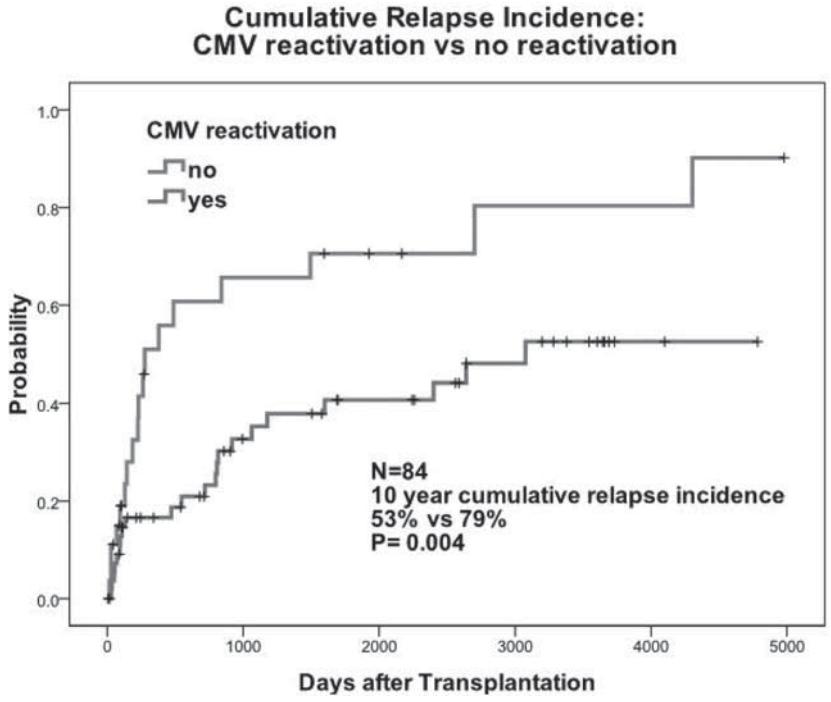
Monitoring of CMV antigenemia and preemptive treatment has diminished mortality from CMV disease. Furthermore, recent studies suggest a favorable "graft-versus-virus" effect from reactivating CMV, reducing relapse of AML after SCT (Elmaagacli *et al* Blood 2011; 118:1402). We retrospectively studied the relationship of CMV reactivation to relapse in patients with CML receiving T cell depleted HLA matched allogeneic SCT. Eighty-four patients with Ph+ CML (mean age 36 years; range 13-69) underwent HLA identical sibling SCT between 1997 and 2008. Sixty-one patients (73%) were in chronic phase, 3 in second chronic phase, 12 in accelerated phase and 8 in blastic phase. Seventy eight (93%) received a myeloablative conditioning regimen which included cyclophosphamide ± fludarabine and total body irradiation. Low dose cyclosporine was used as GVHD prophylaxis. Donor G-CSF mobilized peripheral blood was subjected to a 4-log *ex vivo* T cell depletion. Weekly CMV surveillance was performed until at least day 100 after SCT,

**[P1101]**



using buffy coat pp65 CMV antigenemia before March 2005, and whole blood CMV DNA quantitative PCR thereafter. Positive CMV results were treated with pre-emptive IV ganciclovir or valganciclovir. Foscarnet was substituted in neutropenic patients or refractory cases. Seventy-three (87%) recipients were CMV seropositive before transplant and 68 (81%) received the graft from CMV seropositive donors. CMV reactivation was observed in 56 patients (67%) including 3 with organ CMV disease, 2 pneumonitis and 1 retinitis. At a 6.4 year median follow up, CML relapsed in 42 (50%) patients; 19 hematological and 23 molecular. Relapse free survival was significantly better in CMV reactivating patients (10 year relapse free survival 31% *vs* 12%: P=0.004). Relapse was significantly lower in CMV reactivating patients (10 year actuarial relapse probability 53% *vs* 79%: P= 0.004). In multivariate analysis, only CMV reactivation, donor-recipient sex match (female to male *vs* other) and disease stage (chronic phase *vs* more advanced) remained an independent factor for relapse and relapse free survival (p <0.01, <0.05, <0.001 respectively). We conclude that CMV reactivation in the recipient contributes to a beneficial GVL effect in CML transplant recipients at any stage of disease. The mechanism is unclear but may be related to a bystander activation of innate or adoptive immunity by CMV in the bone marrow reservoir leading to enhanced cytotoxicity of leukemia cells.

**P1102**

**Outcome of allogeneic haematopoietic stem cell transplantation in patients with atypical chronic myeloid leukaemia**

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Atypical chronic myeloid leukemia (aCML) based on the WHO classification is a leukemic disorder that demonstrates myelodysplastic as well as myeloproliferative features at the time of initial diagnosis. In approximately 25-40% of patients (pts), aCML evolves to acute leukemia, whereas the remainder die of marrow failure with a median survival time of less than 20 months with conventional therapy. The only potentially curative therapy is an allogeneic hematopoietic stem cell transplantation (HSCT). A review of our database identified 26 pts (17 male, 9 female) with aCML (Onida score: 0=14 pts, 1=8 pts, 2=4 pts) underwent allogeneic HSCT. The median age at diagnosis was 53 (19-64) years and at transplant 54 (20-66) years. Conditioning therapy included myeloablative regimens in 23 pts (15 TBI based and 8 chemotherapy based) and reduced intensity regimes in 3 pts. Stem cell source was 20 PBSC, 1 CD34 enriched PBSC and 5 BM. Nine pts received stem cell graft from HLA-identical sibling donor (1 twin donor) and 17 pts from matched unrelated donor (7 mismatched). GvHD prophylaxis consisted 21 CSA/MTX, one CSA/MMF, one CSA/antiCD52, one CSA only, and two without (twin and CD34 enriched). Twenty five pts engrafted for granulocytes (ANC>0.5/nl) at median of 21 days (8-31) and 24 pts for platelets at 20 days (11-45), respectively. The most non hematological side effect grade ≥3 was mucositis. Early complete donor chimerism (>95%) was observed in 25/26 pts. Acute GvHD of grade ≥ II occurred in 14 pts and chronic GvHD in 15 pts (10 limited and 5 extensive), respectivity. TRM was observed in 5 cases: sepsis (n=3), MOF/GvHD (n=1), and cerebral toxoplasmosis (n=1). Two pts (twin and transplanted with highly selected CD34+ cells) relapsed after allogeneic HSCT and these pts were successfully retransplanted. 21 pts (80.8%) are still alive at median survival time of 91 months. Our findings suggest that the outcome of allogeneic or syngeneic transplantation in pts with aCML are be feasible without severe TRM and may not be worse than the outcome of transplantation for bcr-abl positive CML.

**P1103**

**Allogeneic stem cell transplantation (allo-SCT) for chronic myeloid leukaemia in the era of tyrosine kinase inhibitors. Analysis of the variables related to relapse and survival**

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Introduction: TKIs are the standard treatment for CML. As TKIs cannot be abandoned without almost 50% of relapse, allo-SCT remains the only curative option and the treatment of choice in advanced phases or failure to TKIs.
Objectives: To analyze overall (OS) and relapse free survival (RFS) after allo-SCT, and to study variables associated to relapse.
Methods: Retrospective analysis to evaluate OS and RFS in CML patients recipients of allo-SCT in our hospital (1995-2010). Analyses were performed with SPSS 15.0.
Results: Between 1995-2010, 125 CML were trasnplantated. In that period of time, only 25 were performed between since 2001. Most part of these patients were previously treated with Interferon and just 18 took TKIs treatment before transplantation. At transplant time, 75% were in chronic phase (CP) with a median age of 37 years (16-59). In 70% of them the donor was familial HLA identical. The 8 years OS was 60% and RFS 55%. Twelve patients died in the early time post-transplant (<120 days). Variables related to higher probability of death were: engrafment failure (HR: 13,9; p=0,003) score EBMT >2 ( HR: 5,2; p=0,02); and acute GVHD ( HR: 5; p=0,04). Previous treatment with TKIs was not significant. The 12 years OS in patients in first chronic phase was 65%.
For relapse analysis, 123 patients were evaluable. 39 (31%) patients relapsed. Relapses were as follows: 39% molecular, 21% cytogenetic, 4% CP, and 36% blast crises (BC). The 8 year-PFS was 55%. Using Cox analysis we identified two variables which were associated with statistically significant lower probability for PFS: not achieving complete chimerism in any moment (HR 0,24: p=0.001), and having advanced phases at transplant (HR: 1,8 p= 0.066). The 12 years RFS in patients in first phase chronic was 58%.
Conclusion: In our study, median OS and RFS post-transplant is about 10 and 8 years respectively. Nearly 33% of patients relapsed and 39% of them did it as BC. The engrafment failure, score EBMT>2 and acute GVHD were significantly linked to higher probability of death; and not to achieve complete chimerism and to have advanced phases at transplant were significantly associated with relapse. These data emphasize the need of a closer control in patients treated with TKI's in order not to delay the transplant. Previous TKI's treatment doesn't seem to affect survival outcomes in allo-SCT.

**P1104**

**Haematopoietic stem cell transplantation for patients with chronic myeloid leukaemia after failure of tyrosine kinase inhibitor therapy**

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Tyrosine kinase inhibitors (TKIs) are the standard treatment for patients (pts) with chronic myeloid leukemia (CML) in chronic phase (CP). Reported results show high rates of complete cytogenetic remissions and excellent long-term survival. Despite these impressive results, approximately 15% of CML CP patients receiving imatinib (IM) therapy will lose their hematologic or cytogenetic response and 1-3% will progress to more advanced phases of disease with inferior survival. For these pts allogeneic stem cell transplantation (HSCT) remains a potentially curative treatment option. Between Jan 2002 and Dec 2010, 23 pts initially diagnosed with CML in CP and treated with IM,

underwent HSCT at our center after IM failure (n=17) (defined as per ELN guidelines) or intolerance (n=6). There were 17 males and 6 females with a median age of 46 years (range, 22-61). Sokal risk groups were high (44%), intermediate (26%), low (22%) and unknown (8%) and the median duration of IM treatment was 12 mos (range 0.7-57). The best response to IM is shown in Table 1. Disease status at HSCT was: CP1 (n=10), CP2 (n=7), CP3 (n=1), AP (n=4) and BP (n=1). The median time from diagnosis to HSCT was 16 mos (range 4-96). Donor source was a HLA matched sibling (n=10), HLA matched unrelated donor (n=8) and a HLA mis-matched unrelated donor (n=5); conditioning was myeloablative in 21 pts; 2 pts had reduced intensity conditioning. All 23 pts engrafted with a median time to neutrophil and platelet recovery of 17 days (range 11-30) and 14 days (range 9-31) respectively. Of the 23 pts, 10 developed grades 2-4 acute graft *vs* host disease (GVHD) and 18 developed chronic GVHD. With a median follow-up of 63 mos (range 15-97) from HSCT, 19 pts are alive and 4 pts have died from relapse (n=2), infection (n=1) and GVHD (n=1). Of the 19 surviving pts, 13 achieved complete molecular remission (CMR) without further therapy (see Table 1), and 6 have relapsed at a median of 13 mos (range 3-54); all of these 6 pts achieved CMR after salvage treatment with a second generation TKI (n=4), DLI (n=1), or withdraw of immunosuppression (n=1). The 5-year event-free and overall survival was 49% and 82% respectively (Figure 1). In conclusion, HSCT is an effective salvage therapy for some pts with IM resistance; however, relapse rates remain high. This highlights the need to try and identify pts early whose response to TKI therapy will not be durable and where HSCT may play a role earlier in their treatment course.

[P1104]

**Table 1** Best response to imatinib and HSCT

| Levels of response | Imatinib n (%) | HSCT n (%) |
|---|---|---|
| No response | 2 (8.7) | 1 (4.3)* |
| Complete Hematologic response | 5 (21.7) | - |
| Minor Cytogenetic response | 3 (13) | - |
| Major Cytogenetic response | 7 (30.4) | 1 (4.3) |
| Complete Cytogenetic response | 2 (8.7) | 2 (8.7) |
| Major Molecular response | 3 (13) | 1 (4.3) |
| Complete Molecular response | - | 18 (78.3) |
| 2nd CP | 1 (4.3) | - |

* died before D100

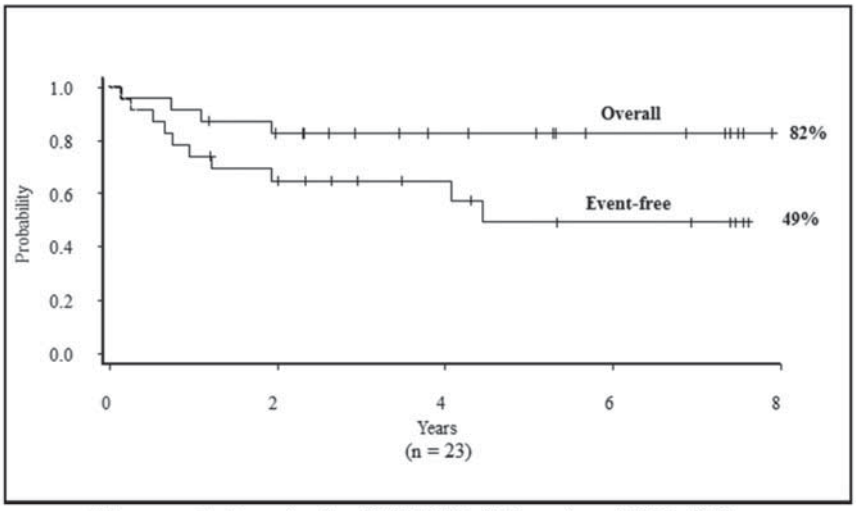


**Figure 1** Survival of HSCT following TKI failure

**P1105**

**Good early and late outcome of CML patients treated with second generation TKI prior to HCT**

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Following ELN guidelines, the patients with chronic myeloid leukemia (CML) Ph(+) resistant to imatinib and second generation tyrosine kinase inhibitors (TKI) are qualified to allogeneic hematopoietic cell transplantation (HCT). Few data are available concerning the toxicity of TKI and final outcome of HCT in TKI-resistant cases.

The retrospective analysis of 20 patients with CML receiving second generation TKI (dasatinib or nilotinib or both) before HCT was performed. 13 patients were in chronic phase (CP), 1 patient in accelerated phase (AP) and 6 patients in blastic phase CML (BP) prior to HCT. 11 patients had matched unrelated donors (MUD) and 9 had sibling donors. Peripheral

blood was used as the source of stem cells in 17 cases and bone marrow in 3 cases. Reduced intensity conditioning (Flu-Mel or FluBu or MelTreoFlu) was given in 7 patients and myeloablative conditioning regimen (BuCy) in 13 patients. All but 1 patient achieved engraftment with median time 19 days (13-43 days). Fatal outcome due to severe transplant-related toxicity developed in 2 (10%) cases: hepatic veno-occlusive disease (VOD) and multi-organ failure. In 6 (30%) patients not significant hepatic toxicity was observed in early post-transplant period. Acute graft versus host disease (GvHD) was diagnosed in 5 (25%) patients (2 in IV grade with fatal outcome) and chronic GvHD in 4 patients.
In the group of 14 patients with CP or AP prior to HCT, 11 patients (78,6%) are still alive and median follow-up is 13 months (2-41 months). All living patients achieved CCyR in early post-transplant period. In 9 of them undetectable BCRABL transcript with a sensitivity of RT-PCR method at $10^4$ is maintained. All of them have much better quality of life (QoL) comparing to time prior to HCT. 1 patient received DLI due to molecular relapse and 1 is in the early post-transplant period. 5 of 6 patients transplanted in BP had fatal outcome early after HCT: 3 due to relapse, 1 because of GvHD and 1of VOD.
Conclusion: Second generation TKI given prior to HCT have no negative influence on early outcome (VOD, GVHD) in patients with CP and AP CML, with fast elimination of the residual leukemic cells measured by RT-PCR and good QoL in late post-transplant period.

**P1106**

**Molecular remission through abrupt interruption of cyclosporine in a patient with blastic crisis of chronic myeloid leukaemia relapsed after allogeneic bone marrow transplantation: evidence of graft-versus-leukaemia effect**

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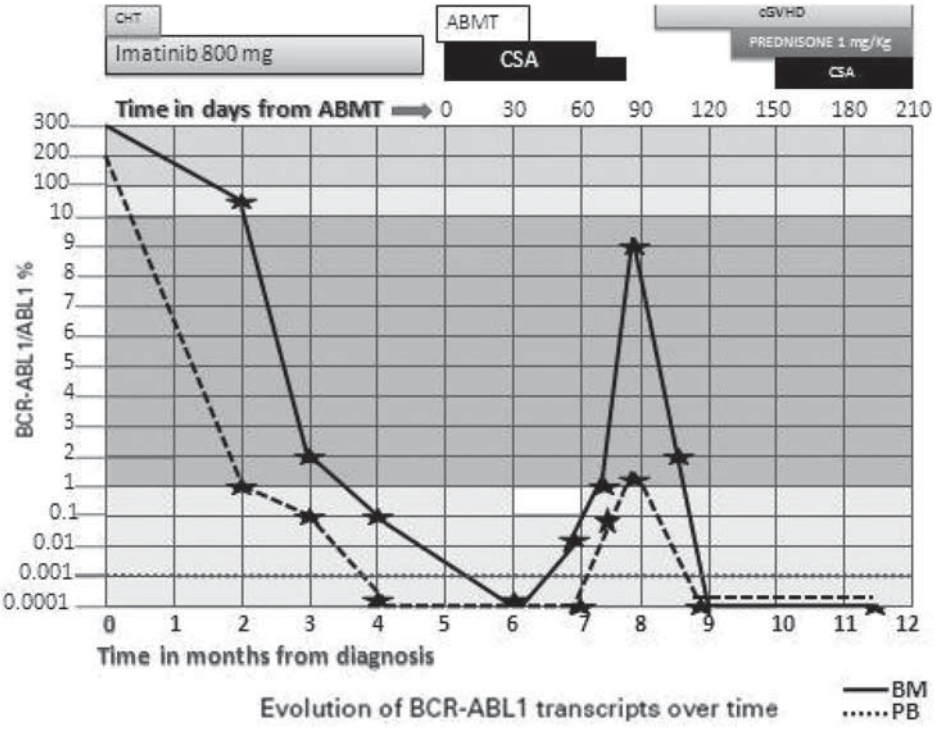
Background: It is known that the graft alloimmune effect can be enhanced by reducing or stopping immunosoppression. Graft-versus-leukemia (GvL) induces by removal of cyclosporine (CSA) have been documented in chronic myeloid leukemia (CML) but these reports do not extend beyond anecdotal examples. We present a case of rapid molecular remission in conjunction with the development of chronic Graft Versus Host Disease (cGVHD) after abrupt interruption of CSA.
Patient: A 38-year-old man and 65 Kg of body weight was admitted to our department with 90% of myeloid blasts in peripheral blood (PB) and in bone marrow (BM), 8% of basophils granulocytes in peripheral blood (PB) and splenomegaly. In flow cytometry the blasts expressed CD13, CD33, and CD34. He was diagnosed with myeloid blastic crisis (BC) of CML despite an absence of a clinical history of a hematologic disorder. Chromosome analysis showed t(9;22)(q34;q11) traslocation in 90% of metaphases. Quantitative RT-PCR analysis on PB sample detected a BCR-ABL–ABL ratio of 255%. The mutation BCR/ABLˆ351NS was evident. The patient underwent one course of idarubicin and cytosine arabinose therapy combined with imatinib 800 mg/day. At month +3 the BCR-ABL transcripts was no detectable on PB samples and positive (ratio 0.14%) on BM sample (Figure 1). At month +4 the patient was treated with myeloablative Busulfan/Cyclophosfamide conditioning and allogeneic BM transplantation (ABMT) from sibling donor. Imatinib was discontinued before ABMT. Neutrophil and platelet engraftment occurred at day +11 and +17 respectively. GVHD prophylaxis consisted of CSA (3 mg/kg/twice daily i.v. from day -1 to +15 and 300 mg/daily orally from day +16) and i.v. MTX (10 mg/Kg/day on day +1 and 8 mg/Kg on day +3, +6 and +11). There was no evidence of acute GVHD. At day +32 from ABMT, the BCR-ABL transcript measured was no detectable on PB and BM samples; at day +70 from ABMT we observed molecular relapse in BM and we reduced the CSA up to a dose of 100 mg/day. At day +82 there was an increase of Ratio (0,2% in PB and 0,8% in BM) (Figure 1). Therefore, the CSA was abruptly suspended. Cutaneous and mucosal manifestation of extensive cGvHD developed and the BCR-ABL transcripts measured on PB and BM samples were no detectable (days +120 from ABMT). cGVHD was treated first with prednisone and then with the addition of CSA.
Conclusion: Our case confirms the existence of GVL effect in CB of CML after abrupt interruption of CSA.

**[P1106]**




Evolution of BCR-ABL1 transcripts over time

**P1107**

**Nilotinib (Tasigna) therapy post allogeneic stem cell transplantation for advanced (>CP1) chronic myeloid leukaemia and Ph+ acute lymphoblastic leukaemia**

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Relapse rate post alloSCT in advanced CML (>CP1) and in Ph+ ALL is high. We assessed (study CAN107AIL03T) whether Nilotinib administration post alloSCT intensifies remission and reduce relapses. 24 pts (M-13, F-11), age 36 years (range, 18-58) participated in the study, advanced CML -17 (BC-10, AP-7), Ph+ ALL-7. 22/24 pts underwent alloSCT (2 are pending) from an HLA-matched sibling (n=11), matched unrelated (n=8) or alternative donor (CB-2, Haplo-1). All, but one, had myeloablative conditioning (Bu/Cy or Flu/Bu-14, TBI/Cy-7). GVHD prophylaxis included CSA and methotrexate or MMF. 21/22 pts engrafted in median day+13 (range, 9-38) with 100% donor chimerism. TRT included mucositis in all pts, encephalitis-2, pericarditis-1, VOD-1 and infection-3. 3 pts died very early post transplant from sepsis and multi organ failure. Acute GVHD ≥ Gr II was observed in 10 pts (III-IV in 5). cGVHD was observed in 15 pts (extensive – 9). 16/22 transplanted pts received Nilotinib (200 mg x 2/d – 10, 300 mg x 2/d – 6) starting at median day +38 (range, 30-158) post alloSCT. 6 pts did not receive Nilotinib post alloSCT due to early death -3, progressive disease -1, severe pancytopenia -1, refusal -1. Nilotinib administration was delayed or dose reduced/stopped in 9 and 7 pts, respectively due to toxicities. All, but 2 pts achieved MMR post alloSCT and 6 of them (CML) converted to CMR following Nilotinib therapy. Kinas mutation (G205E and F359V) were detected only in 1 pt with disease progression. With a median follow up of 16.5 mo (range, 2.5-38) 12 pts are alive while 12 died (Infection -5, GVHD -3, TTP-1, disease progression -3). In 2 pts disease progressed but responded to further therapy. All 6 pts that did not receive Nilotinib died (early death post allo-SCT -3, disease progression -1, GVHD Gr IV -2). Immunological evaluation post Nilotinib administration disclosed no significant change in T, B and NK cell numbers and T cell mitogenic response. Thymic output (TREC) and receptor repertoire (Spectrotyping) analysis indicates continuous thymopoiesis in 8/13 evaluated pts. NK cytotoxic activity against K562 increased in 9/15 evaluated pts. In conclusion, post alloSCT Nilotinib maintenance therapy in extremely high risk pts with advanced CML and Ph+ ALL may prevent relapse and disease progressionand should be recommend as 1) pts achieved MMR and 6 converted to CMR with Nilotinib therapy and 2) only 5/16 pts that received Nilotinib post alloSCT progressed.

**P1108**

**Improved progression-free survival does not translate into better quality of life in chronic lymphocytic leukaemia – results of the randomised EBMT-Intergroup study on the value of autologous transplantation**

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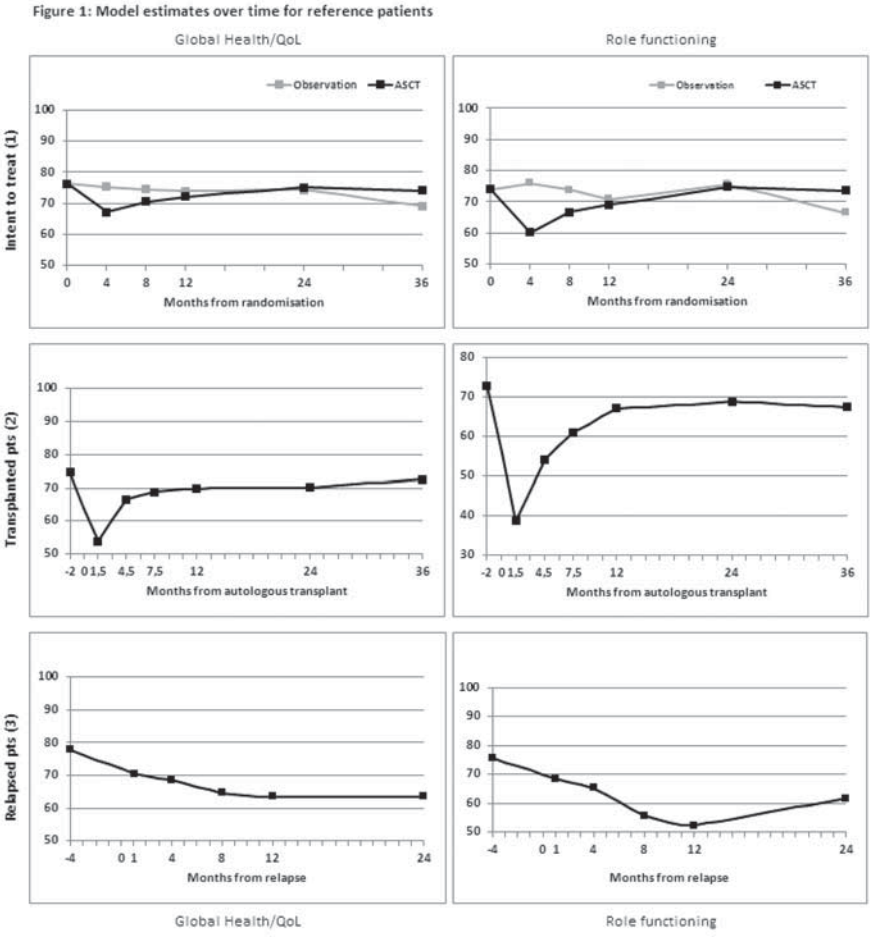
Objective: In chronic lymphocytic leukemia (CLL) medical progress is driven by clinical studies with progression-free survival

**[P1108]**



(PFS) as primary endpoint. The randomized EBMT-Intergroup trial on the value of high-dose therapy and autologous stem cell transplant (ASCT) compared to observation in first or second remission of CLL demonstrated a substantial improvement of PFS without showing improved overall survival for transplanted patients (Michallet, Blood, 2011). Here we report quality of life (QoL) information from that study.
Methods: 222 patients were enrolled into the study and allocated to either ASCT or observation. QoL was assessed with the EORTC QLQ C30 version 3.0. QoL forms had to be completed at randomization and at months 4, 8, 12, 24 and 36. The QoL results are based on data from 186 patients.
QoL-outcomes were analyzed with mixed models (1) according to the intent to treat (ITT) principle, (2) per protocol (PP) with the day of ASCT as time origin and (3) for the subpopulation of relapsed patients with the day of relapse as time origin. Time, the interaction of time and treatment arm (for (1)), remission status at SCT (for (1) and (2)), age, gender and group/country were modeled as fixed effects.
Results: The effects from the ITT model were largest at 4 months and significant for almost all outcomes: e.g., global health status/ QoL -8.1* compared to observation arm; physical -10.3**, role -15.8** and social functioning -15.3**; fatigue +11.9** and appetite loss +15.0** (*p<.05; **p<.01). It seemed to take circa 12 months until resolution of the impact of ASCT. In the observation arm QoL did not decrease significantly in the first two years after randomization but most outcomes were worse after three years. Most likely, this was the result of the increasing percentage of patients with relapse which clearly affected QoL on all subsequent measurements negatively.
Figure 1 shows the estimates of models (1)-(3) for 2 outcomes.
Conclusions: High-dose therapy and ASCT delayed relapse, yet resulted in a substantial but transient decrease of QoL. The net effect in the ASCT arm was an inferior QoL in the first year and comparable QoL until circa three years after randomization. Relapse resulted in a long lasting negative effect on QoL. Although currently ASCT plays no role in the treatment of CLL, this study demonstrates that in general QoL information should be taken into account when treatment decisions are based on an improvement of PFS.

**P1109**

**Tumour-specific immune responses against the receptor tyrosine kinase-like orphan receptor 1 in patients with chronic lymphocytic leukaemia – evidence for immune control?**

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The receptor tyrosine kinase-like orphan receptor 1 (ROR1) is a newly characterized oncofetal antigen in patients with chronic lymphocytic leukemia (CLL). Since a significant proportion of CLL patients never require antitumor therapy, we hypothesized that an autologous tumor-specific immune response is capable of controlling malignant disease. In the current project we analyzed humoral anti-ROR1 immunity in CLL patients. Among sera of 87 untreated patients with CLL, 22 (25.1% with anti-ROR1 IgG antibodies) and 19 (21.8% with anti-ROR1 IgM antibodies) had detectable ROR1-antibodies. IgG subclass analyses showed a predominant IgG1 and IgG3 response. Notably, ROR1-antibodies were capable of recognizing and selectively killing ROR1-expressing CLL cells in complement-mediated and antibody-dependent cellular cytotoxicity (ADCC) assays. In 17 CLL patients receiving allogeneic hematopoietic stem cell transplantation (SCT), 88% exhibited significant titers of anti-ROR1 IgG antibodies. In retrospective analysis we show that untreated CLL patients had a longer progression free survival, if patients' immune system is capable of developing anti-ROR1 antibodies. Our data provide evidence for the existence of a tumor specific humoral immune control in CLL patients.

**P1110**

**Molecular remission in double-refractory CLL treated with sequential FLAMSA induction and HDCY-TBI conditioning followed by allogeneic stem cell transplantation**

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Outcome of patients with CLL double-refractory to fludarabine-based regimens and alemtuzumab has been dismal, without any existing possibility of cure. The median survival has been only few months, and all patients succumb of their disease within one to two years.
We describe a patient with refractory CLL, successfully treated with alloHSCT using sequential therapy including induction with FLAMSA (fludarabine, HD-AraC, amsacrine) followed by slightly reduced myeloablative conditioning.
A 47-year old female was diagnosed to have CLL in 2009, with normal karyotype and unmutated IgH gene. Disease progressed rapidly in January 2010, when blood leukocytes rose above $400x10^9$/l, lymph nodes being widely enlarged. Due to the aggressive course of the disease, treatment was started with fludarabine, cyclophosphamide, and alemtuzumab. Because of liver toxicity, cytotoxic agents were omitted, and treatment was continued with single-agent alemtuzumab up to May 2010, which resulted in PR. ASCT was performed in December 2010. The response remained very short, with a blood lymphocyte rise already within one month after ASCT.
Before the planned alloHSCT in March 2011, a rapid progression of the disease occurred. Blood leukocytes rose to $160x10^9$/l, and massive lymph nodes grew especially in the neck and abdomen. Instead of standard conditioning, FLAMSA induction was administered, with a rapid decrease of tumour burden. After three days rest, conditioning with HD-CY (120 mg/kg) and 8Gy TBI was administered. CyA, ATG, and short MTX were used as immunosuppression (IS). The patient recovered without any primary complications, and had CR and molecular CR in the bone marrow (ASO-qPCR <0.006%, FC <0.005%).
Later she developed grade II skin GVHD at wk +4, and methylprednisolone (MP) was added. IS was tapered from wk 10 posttransplant, but was restarted at 3 months because of grade II skin GVHD. Skin GVHD responded well to MMF and MP but grade IV gut GVHD developed, being steroid-refractory but responding to alemtuzumab. In addition, tacrolimus and everolimus were administered until September 2011 when they all could be tapered off. Nine months posttransplant the patient is in molecular CR, symptomless and without any signs of GVHD.
In conclusion, GvL effect obviously exists in CLL, and may have a curative potential even in the refractory disease. Moreover, sequential conditioning including induction with FLAMSA, as described in AML, seems to be feasible also in severe CLL.

**P1111**

**Donor lymphocyte infusions and/or second treosulfan-based allogeneic stem cell transplantation as salvage treatment for relapsed myelofibrosis after reduced-intensity allografting**

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About 10–30% of patients (pts) with primary myelofibrosis (PMF) experience relapses in first 3 years post-transplant. The prognosis in such cases is unknown and there is no standard therapeutic recommendation for these pts. We here report our

multicenter experience on the use of dose-escalated donor lymphocyte infusions (DLIs) and/or a 2nd HSCT after reduced intensity conditioning in 30 consecutive PMF pts (male, n=21; female, n=9; median age of 57 years (37-67)) with post-transplant relapse (n=27) and graft-rejection (n=3) from 7 transplant centers (Hamburg, Regensburg, Dresden, Düsseldorf, and Hannover in Germany and Tel Hashomer in Israel). A number of 26 pts received a median number of 3 (1-5) DLIs in a dose-escalated mode starting with a median dose of $1.2x10^6$ ($0.3x10^4$-$8x10^6$) up to median dose of $4x10^7$ T-cells/kg ($1x10^7$-$1.3x10^8$). Responses to DLIs were seen in 10/26 pts (39%; molecular CR: n=5; CR: n=5). Incidences of acute (grade II-IV) and chronic graft-versus-host (GvHD) disease were 12% and 36%. There were no cases of GvHD-related deaths. A total of 13 non-responders and 4 pts who did not receive DLIs due to graft-rejection or acute transformation to the blast phase underwent a 2nd allogeneic HSCT after treosulfan (3x12 g/m$^2$)/fludarabine (6 x 30 mg/m$^2$) conditioning from alternative (n=15) or the same (n=2) donor. One pt (6%) experienced primary graft-failure and died. Incidences of acute (II-IV) and chronic GvHD were in 47% and 46%. Responses after 2nd HSCT were observed in 12/15 pts (80%; mCR, n=5; CR, n=4; PR, n=3). The cumulative incidence of non-relapse mortality (NRM) at 1 year after 2nd allograft was 6%, and the cumulative incidence of relapse at 1 year was 24%. The 1-year probabilities of overall (OS) and progression-free survival (PFS) after the 2nd HSCT were 82% and 70%. After a median follow-up of 27 months, the 2-year OS and PFS for all 30 pts was 70% and 67%, respectively. In conclusion, our strategy, including DLIs and 2nd HSCT for non-responding or ineligible pts, is an effective and well-tolerated salvage approach for relapsing after reduced-intensity allograft PMF pts. Pts with residual fibrosis post-transplant as well as those with evidence of molecular progression, monitored by measurement of JAK2V617F- or MPL-mutation load, are suggested to have a benefit from the dose-escalated administration of DLIs. In pts with failure to DLIs the prognosis may be improved by the use of a 2nd, toxicity-reduced allograft.

**P1112**

**Significance of prognostic scores, risk factors and chronic graft-versus-host disease on outcome after allogeneic haematopoietic stem cell transplantation for myelofibrosis**

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Primary or secondary myelofibrosis (MF) is a chronic myeloproliferative stem cell disorder curable exclusively by allo-HSCT.
76 patients (pts) (40 male, 36 female; median age at transplant=51 years) with primary (n=47), post-polycythemic (n=12) or post-thrombocythemic (n=17) MF underwent allo-HSCT after myeloablative conditioning containing fractionated total body irradiation (TBI) (n=45), chemotherapy regimen (n=26) or reduced intensity conditioning (n=5). Donors were HLA-identical (n=27) or mismatched (n=3) siblings and matched (n=33) or mismatched unrelated (n=13). Transplants consisted of unmanipulated peripheral blood stem cells (n=68), bone marrow (n=6) or highly purified CD34+ cells (n=2). GVHD-prophylaxis was performed with CSA + MTX (n=46), 17 pts. received anti-thymocyte-globulin (ATG) or alemtuzumab (n=13). Advanced (n=35) and non-advanced disease stages were categorized as described earlier (Ditschkowski *et al*., BMT 2004). Dupriez- and EBMT risk score, dynamic international prognostic scoring system (DIPSS) and age-adjusted (aa) DIPSS were generated for each pt. prior to HSCT.
After a median follow-up of 55 months among surviving pts., 1-year TRM was 22%, primary graft-failure occurred in 4 pts (5%) and relapse was observed in 17% at median 6 months post-transplant. 5-year overall survival (OS) was 45%, 5-year relapse-free survival (RFS) 50%. Incidence of chronic GVHD was 68% at 2 years after HSCT. Estimated RFS was 65% for chronic GVHD (n=44) at 5 years *vs*. 24% for pts. without cGVHD (p<0.0001). 5-year probability of RFS was assessed 59% for non-advanced *vs*. 39% for advanced disease stages. In the univariate model chronic GVHD (p<0.0001), cytoreductive pre-treatment (p=0.015), circulating blasts (p=0.026), advanced/non-advanced disease (p=0.011), aaDIPSS (p=0.044) and DIPSS (p=0.032) showed significant influence on OS. The multivariate model approved cGVHD (HR, 0.14; 95% CI 0.06 to 0.29), DIPPS (HR, 2.1; 95% CI 1.2 to 3.6) and disease stage (HR, 2.5; 95% CI 1.2 to 4.9) to significantly discriminate for OS (p<0.0001, p=0.007, p=0.01). Graft type, donor, gender constellation, Dupriez score, EBMT risk score, grade of marrow fibrosis, recipient age, JAK2 mutation status, splenectomy, conditioning, immunosuppression, HLA-match, abnormal karyotype, and acute GVHD had no impact on OS.
Our data demonstrate that cGVHD is a pivotal factor for post-transplant survival. The feasible applicability of DIPPS in the context of HSCT could be validated.

**P1113**

**Chronic eosinophilic leukaemia with erythroblastic proliferation and the recurrent chromosomal translocation t(8;9)(p22;p24) with PCM1-JAK2 fusion gene – a distinct clinical, pathological and genetic entity successfully treated with alloHSCT**

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Background: Myeloid and lymphoid neoplasms resulting from translocation t(8;9)(p21-23;p23-24) fusing the human autoantigen pericentriolar material 1 (PCM1) gene and the janus-activated kinase 2 (JAK2) gene are very rare disorders.Until now 17 patients with PCM1-JAK2 fusion gene were described.
Case Report: In a 22-year-old woman in 28th week of gestation idiopathic hypereosinophilic syndrome was diagnosed according WHO in December 2008. Pregnancy was terminated at 38th week, delivering healthy newborn. This woman was treated with INFalpha; and hydroxyurea. In March 2010,the BM was still hypercellular (90%) with proliferation of eosinophils and granulocytes. Large nodules composed of erythrobla sts,proerythroblasts and some immature cells were observed. Immunohistochemical staining demonstrated erythroid nature of these cells. Moreover, increase in reticulin fibrosis was observed (grade 2). From September 2010 significant anemia developed. The patient was qualified for alloHSCT from the HLA-matched sibling, but because of the pregnancy of the donor, the procedure was postponed.In March 2011, before alloHSCT, karyotype revealed translocation t(8;9)(p22;p24) and chimeric PCM1-JAK2 detected by RT-PCR in BM sample. Sequencing confirmed exon to exon in-frame fusion between PCM1 exon 36 and JAK2 exon 9 with the breakpoint at position 6281-6282 and 1710-1711, respectively. In trephine biopsy, peritrabecular nodules substituted about 50% of marrow cellularity and exhibited increased number of immature erythroid cells, expressing hemoglobin A, CD71 and p53, with proliferation fraction about 90% in Ki67 stain. Blasts markers CD34, CD117 were negative. Reticulin fibrosis was significant (grade 3). Previous diagnosis of idiopathic hypereosinophilic syndrome was verified to chronic eosinophlic leukemia, not otherwise specified (CEL, NOS) with erythroblastic proliferation of unclear significance. In May 2011, alloHSCT was

performed with conditioning BuFlu (busulphan i.v. 3,2 mg/kg a day, days -7 to -4 and fludarabine 30mg/m2 a day, days -7 to -3). Prophylaxis of GvHD included CsA/MTX. On day +180, 100% donor chimerism was confirmed in STR-PCR.Cytogenetic analysis of BM aspirate revealed normal karyotype. No GvHD was observed.
Conclusions: Our report is unique as it illustrates diagnostic difficulties within 2.5-year follow-up and presents the study of evolution of this rare disease.Such detailed case descriptions are important,as they will greatly facilitate constructing definition criteria in the future and help to find the best therapeutic option.

## Myelodysplasia

**P1114**

**Feasibility of hypomethylating therapy before allogeneic stem cell transplantation as bridging therapy in patients with myelodysplastic syndrome**

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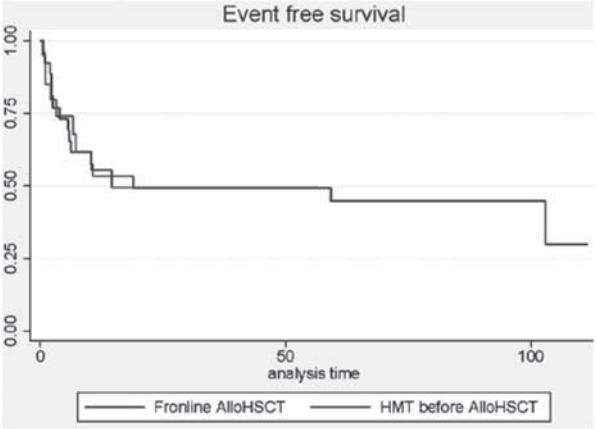
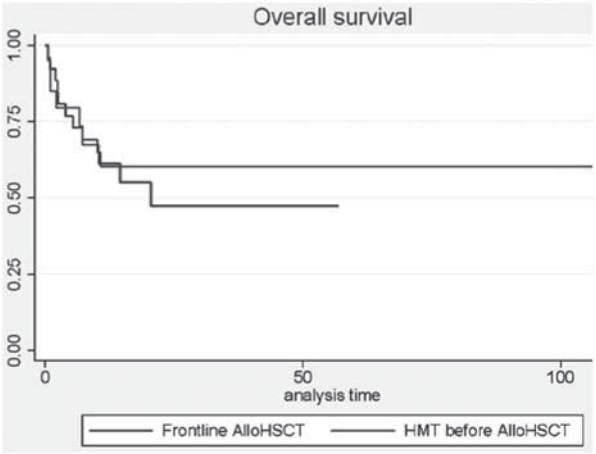
Allogeneic hematopoietic stem cell transplantation (HCT) is the only curative modality for most patients with myelodysplastic syndrome (MDS). We assessed the transplant outcomes for 47 adult MDS patients, median age 45 (21-67 years), undergoing allogeneic hematopoietic stem cell transplantation (HSCT) at Seoul National University Hospital between 2001 and 2011. Among 47 patients, 26 (55%) received frontline HSCT, whereas 20 (43%) received hypomethylating therapy (HMT) before HSCT as bridging therapy. By WHO criteria 26 (55%) had refractory anemia with excess blast (RAEB-1 or 2), 8 (17%) had refractory cytopenia with multilineage dysplasia (RCMD), and remaining 13 (18%) had refractory cytopenia with unilineage dysplasia (RCUD), or myelodysplastic syndrome-unclassifiable (MDS-U). Graft source was full matched related in 27 (57%), unrelated peripheral blood in 14 (30%), and partially mismatched related in 3 (6%), and unrelated in 3 (6%). The conditioning regimen was 17 (36%) myeloablative (MA) and 30 (64%) nonmyeloablative (NMA) regimens. The cumulative incidences of neutrophil engraftment, grade 2 to 4 acute GVHD, chronic GVHD, and transplantation-related mortality after HSCT, were 98%, 48%, 47%, and 24%, respectively. After a median follow-up time of 56.8 months, event-free survival (EFS) and overall survival (OS) rates were 29% and 53%, respectively, for all patients.
Comparing OS & EFS between frontline HSCT group and HMT bridging group, the survival rates were not significantly different (log-rank test p=.58 & p=.98 respectively).
In conclusion, HMT followed by alloHCST was feasible and effective treatment strategy for patients with MDS who need considerable time until alloHSCT.

**[P1114]**




**P1115**

**Allogeneic haematopoietic stem cell transplantation for chronic myelomonocytic leukemia: a report from the Société Française de Greffe de Moelle et de Therapie Cellulaire**

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Background: This retrospective study aimed at determining prognostic factors for OS after allo-SCT in a group of consecutive 73 CMML patients (pts) reported to the SFGM-TC registry between 1992 and 2009.
Methods: Pt data at diagnosis and at transplant (tx), including WHO classification, IPSS in pts with WBC <13G/L, and for CMML with WBC >13 G/l, palpable splenomegaly (SPM), Hb<10g/dl, Plts<100 G/l, marrow blasts >5%, abnormal karyotype, extramedullary disease (GFM score), interval between diagnosis and allo-SCT, and prior treatment were analyzed.
Results: Pt characteristics at diagnosis were: M/F 49/26, median age 53 yrs (range, 27-66). 36% pts had palpable SPM, 70% WBC>13×$10^9$/L. 48/12/9 pts had good/int/poor risk karyotype according to IPSS, including normal (n=47), monosomy 7 (n=7) and tris 8 (n=5). 61% pts had CMML1, and 39% CMML-2. Of the 22 pts with WBC<13G/l, 6/1 had int-2/high risk IPSS, while of the 45 pts with WBC >13G/l, 37 had at least 2 poor prognostic factors of the GFM score. Before allo-SCT, 26 pts had received intensive chemotherapy (CT), 21 low dose CT (18 HY, 3 VP16) and, 6 hypomethylating agents. 32 pts developed infection between diagnosis and allo-SCT. Median interval from diagnosis to allo-SCT was 10.6 mo (range 2.8-80). At time of allo-SCT, 15 pts

had responded to AML like therapy, while 42 pts were treatment failure or in relapse, or had not been treated, including 5 AML progressions. 19 pts still had palpable SPM before allo-SCT. The donor was an HLA-identical, unrelated and haploidentical sibling in 41/31/1 cases respectively (resp.). 30 and 43 pts received MAC and RIC regimen resp. With a median follow-up of 23 mo (1-145), grade 0-1 acute GVHD developed in 23 pts, grade 2-4 in 21 pts. Chronic GVHD was present in 25 pts (limited: 15, extensive: 10; cum incidence: 37% at 3 yrs). The 2- and 3-yr OS were 42% and 32%, resp. The 3-yr cum incidence of NRM was 36%. The 3-yr RFS was 30%. OS was not influenced by the disease status at allo-SCT, including CMML1 *vs* CMML2, IPSS, the number of prior treatments, HLA matching, cGVHD. Palpable SPM at tx was a negative prognostic factor for OS (2-yr OS: 52% *vs.* 28%, p=0.03). 2-yr DFS was better after 2004 (57% *vs* 19%, p=0,016). In multivariate analysis, the strongest prognostic factor for OS was palpable SPM at tx (HR=0,48 95% CI: 0.24-0.98; p=0.042). Conclusion: Palpable SPM at tx and tx before 2004 were the only independent negative prognostic factors for OS.

**P1116**

**Allogeneic stem cell transplant for myelodysplastic syndromes: results of 291 patients from Spanish MDS Registry. Subanalysis on low-risk patients**

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The only curative approach for patients with MDS is allogeneic hematopoietic stem cell transplantation (HSCT). We have retrospectively analyzed the results of 291 MDS patients receiving an allogeneic HSCT in Spain. An IPSS score, defined as IPSS-pretrasplant was calculated at the time of the trasplant setting, in order to analyze their impact on transplantation evolution.

Results: The median age at transplant was 47 years old, range (14-72), 68% of patients received mieloablative conditioning regimen and all but eleven patients engrafted (96%). Regarding GVHD, incidence of acute (aGVHD) and chronic (cGVHD) GVHD were 47% and 55%, respectively. After a median follow-up of 2.6 years (95% CI 0.7- 4.5 years), OS was 33% and EFS was 32%. Trasplant related mortality (TRM) was 41%, relapses and infection were the most frecuent cause of death (66%). Regarding multivariate analysis for OS disease status at trasplant [(partial response (PR) and no response (NR), HR 1.96; CI (1.143-3.39), p=0.015 for PR and HR 2.33; CI (1.393-3.39), p=0.001 for NR)], high risk cytogenetic [(HR of 1.816; CI (1.216-2.711), p=0.005] and IPSS pre-trasplant [HR of 1.5; CI (1.056-2.245), p=0.025] retained statistical influence on survival. Variables with statistical significance for EFS were these variables along with aGVHD grades 3-4 [(HR of 1.942; CI (1.025-3.678), p=0.04)] and no developing cGVHD (HR of 1.676; CI (1.058-2.657), p=0.002)].

Regarding low risk patients, an specific analysis was performed for 43 patients who retained low risk characteristics at the time of the trasplant. Bone marrow was the source in half of patients and 84% of patients received myeloablative conditioning regimen. All but 6 patients engrafted (86%), incidence of aGVHD was lower in this subset of patients (40%, with 5% of patients developing grades 3-4) and cGVHD was 53% with 23% of patients developing extensive cGVHD. After a median follow-up of 12.6 years, OS was higher than in the whole group, 43%, TRM was lower (28%). Infections were the most frecuent cause of death (44%). Multivariate analysis, is shown in Graphic 1.

Conclusion: Further analysis must be done to improve outcome in the trasplant setting of MDS patients, diferent pre-trasplant variables seems to amilorates the benefit of this procedure and another strategies should be considerer. Regarding low risk patients, due to the lower relapses, strategies improving outcome must reduce mortality, the use of RIC regimens could benefit this patients.

**[P1116]**

MULTIVARIATE ANALYSIS OF PROGNOSTIC PARAMETERS AMONG LOW RISK PATIENTS

| Variable | HR (95% CI) | p |
|---|---|---|
| **Prognostic parameters for overall survival** | | |
| Cytogenetics risk group | | |
| Good | Reference | |
| Intermediate | 4.54 (1.13 – 18.24) | 0.03 |
| Poor | 11.27 (2.12 – 59.93) | 0.004 |
| Stem cell source | | |
| Bone Marrow | 5.77 (1.57 – 21.14) | 0.008 |
| GVHDc | | |
| Yes | 11.00 (2.36 – 51.17) | 0.002 |
| **Prognostic parameters for event free survival** | | |
| Cytogenetics risk group | | |
| Good | Reference | |
| Intermediate | 4.62 (1.15 – 18.51) | 0.03 |
| Poor | 11.45 (2.15 – 60.90) | 0.004 |
| Stem cell source | | |
| Bone Marrow | 5.89 (1.61 – 21.51) | 0.007 |
| GVHDc | | |
| Yes | 11.00 (2.36 – 51.25) | 0.002 |

**P1117**

**Allogeneic stem cell transplantation in patients with myelodysplastic syndromes/secondary acute myeloid leukaemia: A single-centre experience**

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Objectives: Myelodysplastic syndromes (MDS) are a heterogeneous group of stem cell disorders characterized by bone marrow dysplasia, peripheral cytopenia, and an enhanced risk to transform to acute myeloid leukemia (AML). In most patients, treatment options are limited to supportive care and palliative cytoreduction. However, in a group of patients, intensive therapy can be offered. The only established curative treatment approach for these patients is haematopoietic stem cell transplantation (SCT). Methods: In the present study, we retrospectively analyzed a cohort of 60 adult patients (33 males, 27 females) with MDS (n=28) or MDS transforming into secondary AML (n=33), who underwent SCT at our institution between 1988 and 2010. Twenty-eight patients had an HLA-identical related transplant donor, and 32 had an HLA-matched unrelated donor. The median age at time of SCT was 44 years (range: 18 to 68 years). According to the WHO classification, 4 patients had RA, 1 RARS, 3 RCMD, 1 RCMD-RS, 6 RAEB-1, 12 RAEB-2, 1 CMML, and 32 had AML following RAEB at SCT. Conditioning consisted of chemotherapy plus total body irradiation (55/60 patients) or chemotherapy alone (5/60 patients). Graft versus host disease (GvHD) prophylaxis consisted of a combination of low-dose methotrexate and cyclosporine A (37/60 patients) or cyclosporine A plus mycophenolat mofetil (23/60 patients).
Results: Patients were followed up with a median observation time of 16 months (range: 1-218). Currently, 34 patients (57%) are alive at. Of the 26 patients who died, post-transplantation relapse occurred in 12 patients, and14 patients died of treatment-related causes (multi-organ failure, sepsis, haemorrhage).
Conclusions: In summary, a substantial number of patients with MDS achieve long term disease-free survival after SCT. In our small cohort of patients, the overall outcome and survival after SCT was independent of IPSS risk categories or the WHO classification. However, we identified co-morbidity - assessed by HCT-CI - as a significant adverse prognostic variable in our MDS patients, confirming previous data. Therefore, we believe that it is important to use score systems/prognostic factors to optimally predict survival in MDS patients who are considered candidates for SCT.

**P1118**

**Allogeneic haematopoietic cell transplantation in myelodysplastic syndromes: a single-centre experience**

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Indications, timing of HCT in MDS and best pre-transplant treatment, are debated. We thus investigated local practices of indications and transplant outcomes, from the referral perspective. Outcomes of MDS patients, who underwent HCT between 1994 and 2011 in our center, were retrospectively analyzed. Thirty-one consecutive patients allotransplanted for MDS were enrolled, with a median age of 52 y (5-62) and M/F ratio 1.2. Disease status at HCT was RA 6%, RARS 3%, RAEB 32%, RAEB-T 16%, MDS with myelofibrosis (MF) 16%, sAML 26%. 23% of patients had treatment-related MDS. According to IPSS-R (Greenberg *et al*, 2011), karyotype (KT) was good, intermediate, poor and very poor in 39%, 23%, 13% and 10% of cases, respectively while 16% of patients had monosomal KT. Median interval from diagnosis to HCT was 9.5 months (1-91). Before transplantation, induction chemotherapy (IC) and azacitidine (AZA) were administered in 48% and 20% of the whole cohort and 93% and 12% of patients with sAML/RAEB-T, respectively. CR rate after IC was 48% and median response duration 11 months. After a median of 5 cycles of AZA, 50% and 33% of patients achieved partial response and hematological improvement, respectively. Percentage of marrow blasts at HCT was <10 and ≥10% in 50% of patients each. Donors were siblings for 71% of patients and the rest were alternative transplants. Myeloablative (MA, mainly BUCY) and reduced-intensity conditioning (RIC, mainly FLUBUATG) were administered in 63% and 37% of patients, respectively. The 5-y overall survival (OS), cumulative incidence of relapse and non-relapse mortality (NRM), were 20%, 35.6% and 38%, respectively. Prognostic factors for longer OS were % of marrow blasts <10% (33 *vs.* 5 months with ≥10%, P=0.01) and, as a trend, CR (24 *vs.* 6 months without CR, P=0.17). Monosomal and very poor KT adversely affected relapse mortality (HR 3.7, P=0.07; HR=2.6, P=0.002). No predictive factor was identified for NRM among age, sex, donor and conditioning type. All but one of the survivors had undergone HCT less than two years after MDS diagnosis. In conclusion, percentage of marrow blasts was the strongest prognostic factor for overall survival after HCT, inciting to cytoreductive treatment in advanced MDS and/or early referral. Adverse outcome of patients with newly defined very poor karyotype urges for alternative HCT modalities. The choice of RIC *vs.* MA conditioning should be based on personalised decisions, mainly comorbidity.

**P1119**

**Sweet's syndrome in myeloproliferative syndrome resolved after allogeneic stem cell transplantation**

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Background: Sweet's syndrome (or acute febrile neutrophilic dermatosis) consists of the abrupt onset of red, tender, cutaneous plaques on the face, extremities, and upper trunk, accompanied by fever, malaise, and neutrophilic leukocytosis. Histologically, there are distinctive, dense, dermal infiltrates of neutrophils. Response to systemic steroids is typically dramatic. Characteristically, Sweet's syndrome occurs in the clinical setting of a disease-related myeloid malignancy or is medication triggered.
Case Report: A 59-year old male patient presented with multiple red, tender, cutaneous plaques on the face, the extremities and the trunk. He had continuous fever preceded by many months of active arthritis of large joints. He required high doses of steroids to control his symptoms. Skin biopsy revealed neutrophilic infiltration but no signs of malignancy or vasculitis. The patient developed heart failure caused by an atrio-ventricular block requiring implantation of a pacemaker. Heart biopsy revealed patchy neutrophilic infiltrations. The general condition increasingly deteriorated with fever, malaise and peripheral neutrophilic leukocytosis. A diagnosis of Sweet's syndrome was made.
In a search for an underlying hematologic malignancy, a bone marrow examination was performed and indicated a myeloproliferative disorder unclassifiable (MPD). A JAK2 mutation was negative and BCR-ABL could be excluded by molecular diagnostics. Cytogenetics were normal. A cytoreductive treatment with hydroxyurea was initiated with clinical slight improvement of the skin manifestations. Finally, allogeneic bone marrow transplantation after fludarabin/busulphan conditioning was performed using stem cells from a 10/10 HLA-identical unrelated donor. GVHD prophylaxis consisted of ATG, methotrexate and cyclosporine. He achieved full donor chimerism without clinically manifest GvHD. Manifestations of Sweet's syndrome and arthritis disappeared within two weeks following transplantation and remained in complete remission.

Conclusion: Our report indicates a close association between Sweet's syndrome and myeloid malignancies. Ultimately, this may even require allogeneic stem cell transplantation as in our patient with myeloproliferative dysorder.

## Lymphoma

**P1120**

**Identification of prognostic factors predicting outcome in Hodgkin's lymphoma patients relapsing after autologous stem cell transplantation and post-relapse treatment outcome**

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Objectives: To evaluate outcome of patients with relapsed Hodgkin's lymphoma (HL) after high-dose chemotherapy followed by autologous stem-cell transplantation (ASCT) and to distinguish different risk groups using identified prognostic factors.
Patients and Methods: We reviewed 511 adult patients registered in the European Group for Blood and Marrow Transplantation (EBMT) and the Gruppo Italiano Trapianto di Midollo Osseo (GITMO) databases between 1996 and 2005. Tandem ASCT and patients receiving only palliative care after relapse were not included in the study.
Results: Median time from ASCT to relapse or progression was 7 months (range, 1-78). Treatment following ASCT failure consisted on conventional chemotherapy and/or radiotherapy in 294 patients (64%), second ASCT in 35 (8%), and allogeneic SCT in 133 (29%). After a median follow-up of 49 months, overall survival (OS) was 32% at 5 years. OS at 4 years for patients treated with allogeneic stem cell transplantation with reduced-intensity conditioning regimens was 48% in comparison with 32% for those receiving chemotherapy/radiotherapy. In multivariate analysis, independent risk factors for OS were early relapse within the first 6 moths after ASCT, stage IV, bulky disease, poor performance status, and age > 50 years at relapse. For patients with no risk factors OS at 5 years was 62% compared with 37% and 12% for those having 1, and >2 factors, respectively. This score was also predictive for outcome in each group of rescue treatment after ASCT failure.
Conclusion: In the EBMT database, most HL patients with ASCT failure are treated with chemo-radiotherapy and some of them with a second transplantation. Early relapse, stage IV, bulky disease, poor performance status and age > 50 at relapse are relevant factors and could be used to guide the choice of treatment for individual patients and to understand the results of novel different therapeutic approaches.

**P1121**

**Early mortality after allogeneic stem cell transplantation in adult T-cell leukaemia/lymphoma patients**

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Background: Adult T-cell leukemia/lymphoma (ATLL) is a highly aggressive hematological malignancy caused by a human T-cell lymphotropic virus type 1 (HTLV-1). Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is increasingly used as a curative option. However, 3-year overall survival rate is low with approximately 30% and incidence of non-relapse mortality is so high that incidence of early mortality would be increased. Therefore, it is necessary to consider its indication. Here we clarify the factors of early mortality within 100 days after allo-HSCT retrospectively in our single institute located in endemic area of ATLL.
Methods: There were 59 ATLL patients undergone allo-HSCT at Imamura Bun-in Hospital from June 1998 to March 2010. We analyzed the clinical characteristics of 13 patients died within 100 days after allo-HSCT compared with 46 patients alive over 100 days retrospectively.
Results: In 13 patients (8 male, 5 female), median age was 52 (range: 40-59) years. All diagnosed as acute type. Disease status at HSCT was 3 CR and 10 non-CR (SD 2, PD 8). HCT-CI score was 0 in 3 patients, 1 and 2 in 7 patients, and over 3 in 3 patients, respectively. EBMT score was 2 in 1 patient, 4 in 2 patients and over 5 in 10 patients, respectively. Six patients received BMT, 6 PBSCT, and 1 CBT, respectively. Eight patients had received CST and 5 RIST. HLA matching was 6/6 in 5 patients, 5/6 in 5 patients, and 4/6 or 3/6 in 3 patients, respectively. Four patients of 11 complicated with grade 0-I, 7 grade II-IV. The causes of death were 4 TMA, 3 disease progression, 3 aGVHD, 2 sepsis, 1 GI bleeding, respectively. In univariate analysis, significant factors contributed to early mortality were HLA mismatched donors ($p<0.001$), high value of soluble IL-2 receptor (sIL-2R) at HSCT ($p<0.001$) and EBMT score over 5 ($p=0.03$). PD status at HSCT ($p=0.12$), HCT-CI score over 3 ($p=0.47$), RIST ($p=0.85$), grade II-IV of aGVHD ($p=0.46$), and high value of LDH ($p=0.59$) were not contributed to incidence of early mortality.
Conclusion: HLA disparity, high value of sIL-2R at HSCT and EBMT score over 5 contributed to incidence of early mortality. Our results suggest that it should be considered not only disease status including value of sIL-2R at HSCT, but also HLA matching and EBMT score when planning to undergo allo-HSCT for ATLL patients.

**P1122**

**Allogeneic haematopoietic stem cell transplantation for non-Hodgkin lymphoma. A single-centre experience**

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Background: For most NHL allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains a last option because of a high efficacy of autologous HSCT in relapsed chemosensitive disease and historically high NRM reported with the former. However, for some NHL allo-HSCT represents a unique potentially curable treatment. Evolution of conditioning regimens with shift to the reduced intensity (RIC), amelioration of supportive care, GvHD prevention and treatment have lead to decreased NRM rates and subsequent increased allo-HSCT usage in NHL. We report here a single center experience of allo-HSCT in NHL patients treated at the transplantation unit of University Hospital of Geneva (HUG).
Materials and Methods: Study included 49 pts with different NHL types received allo-HSCT at HUG between 11/1984 and 08/2011. All pts had relapsed disease after the best conventional therapy available. Pts baseline and alloHSCT characteristics are presented in the tables.
Impact on OS, current DFS (cDFS, rescue after DLI) and NRM was assessed in univariate analysis for: i) prior auto-HSCT, ii) disease status at alloHSCT iii) Karnovsky indx < 80% iv) graft type v) BM *vs* PB stem cell source vi) T-depletion by Alemtuzumab *in vitro* vii) conditioning type: RIC *vs* MAC viii) TBI usage ix) acute GvHD, including aGvHD more than grade 2 x) chronic GvHD, including extensive form xi) DLIs in post alloHSCT xii) T *vs* B-cell origin of NHL xiii) indolent *vs* aggressive NHL.
Results: Median follow up was 3,7 years (0,3-17,9 years). Median age was 43 (range, 4-59). 5 years OS, cDFS and NRM were 59±15%, 57±15% and 23±14% respectively.

[P1122]

| | | |
|---|---|---|
| Gender (M:F) | 35:14 | 71:29 |
| **NHL types** | | |
| Indolent | 32 | 80% |
| Aggressive | 17 | 20% |
| B-cell | 43 | 87% |
| T-cell | 6 | 13% |
| Diffuse large B-cell lymphoma | 14 | 28% |
| Mantle cell lymphoma (MCL) | 12 | 25% |
| CLL | 11 | 20% |
| Follicular | 5 | 10% |
| Lymphoplasmocytic | 1 | 2% |
| angioimmunoblastic | 2 | 4% |
| T-prolymphocytic leukemia | 2 | 4% |
| Extranodal NK/T cell type | 1 | 2% |
| Enteropathy associated | 1 | 2% |
| **Disease status at alloHSCT** | | |
| CR | 26 | 53% |
| PR | 14 | 29% |
| SD | 2 | 45% |
| PD | 8 | 16% |
| 1st CR | 4 | 8% |
| >1st CR | 22 | 45% |
| **Patients status pre alloHSCT** | | |
| Karnovsky index ≤80%: yes | 7 | 14% |
| Previous autoHSCT: yes | 17 | 35% |

| | | |
|---|---|---|
| **Conditioning** | | |
| RIC | 21 | 43% |
| MAC | 28 | 57% |
| TBI in conditioning: yes | 30 | 61% |
| **Grafts characteristics** | | |
| HLA-identical sibling | 33 | 67% |
| Matched unrelated (MUD) | 9 | 18% |
| Mismatched unrelated | 5 | 10% |
| Twins | 2 | 5% |
| **Post alloHSCT** | | |
| Acute GVHD (aGvHD): yes | 26 | 53% |
| aGVHD ≥ Grade2: yes | 19 | 39% |
| Chronic GvHD (cGvHD): yes | 16 | 33% |
| Extensive cGvHD: yes | 10 | 20% |
| Donor lymphocytes infusions (DLIs): yes | 13 | 27% |
| Relapse/progression (Rel/PD) | 18 | 37% |
| **Status at last observation** | | |
| Death | 18 | 37% |
| CR | 28 | 57% |
| Rel/PD | 3 | 6% |
| **Cause of death** | | |
| NHL | 10 | 20% |
| AlloHSCT-related | 8 | 16% |

Statistically significant parameters negatively influencing OS were: Karnovsky <80% (p<0,0001), PBSC as graft source (p=0,048), T-cell origin (p=0,024), not CR/PR status at HSCT (p=0,051). The only B-cell origin was associated with longer cDFS (p=0,027). NRM was significantly higher in pts with Karnovsky <80% (p=0,048).
Conclusions: Our analysis of mixed-types NHL cohort confirms efficacy of allo-HSCT in heavily pretreated/refractory patients showing promising 5 years OS and acceptable NRM rate. In this series, OS was found to be independent of NHL type and cDFS was not influenced by type of conditioning regimen or graft T-cell depletion. A proportion of pts relapsing after allo-HSCT can be rescued by DLIs with achievement of prolonged CR.

**P1123**

**Allogeneic haematopoietic stem cell transplantation in patients with non-transformed follicular lymphoma grades I-IIIA: data provided by the German Registry for Stem Cell Transplantation**

*F. Heinzelmann, D.W. Beelen, G. Ehninger, J. Finke, C. Mueller, H. Schrezenmeier, H. Ottinger on behalf of the DRST Follicular Lymphoma Study Group*

Background: The aim of this retrospective study was to analyze the clinical outcome of patients with FL grades I-IIIA treated by allogeneic HSCT and to define subgroups which benefit most from allogeneic HSCT.
Patients and Methods: In the DRST database 146 patients (male: 89; female: 57) with FL were identified who underwent allogeneic HSCT between 1998 and 2007. 90/146 patients were pre-treated with autologous HSCT. Median age at allogeneic HSCT was 48 years (range 29-70). 110/146 patients were either in complete (CR, n=36) or partial (PR, n=74) remission whereas 33/146 patients had refractory disease (RD). Median time from diagnosis to allogeneic HSCT was 44 months (range 7-205). Median time interval between autologous and allogeneic HSCT was 23 months (range 1-165). The conditioning regimen prior to allogeneic HSCT was reduced intensity (RIC, 96/146; chemotherapy-only: n=55; TBI-based: n=41) and myeloablative (MA, 50/146; TBI-based: n=24; chemotherapy-only: n=26). Median donor age was 40 years (range 21-69; female: n=54; male n=92). 81% of the patients had fully HLA-matched donors either siblings (42%) or unrelated (39%). At the present state of evaluation, univariate statistical analysis was performed.
Results: Engraftment was seen in 95% of the cases (median: 13 d). Incidence of acute GvHD was 74% in the MA group and 55% in the RIC group (p=0.04). Extensive chronic GvHD was more common in patients with elderly donors (p=0.016). For the entire study population, estimated 5-year overall survival (OS) was 52%. Of 51 evaluable patients surviving more than 5 years, only one patient relapsed (2%). Cumulative 100d TRM was 16%. Limited chronic GvHD (p=0.015) and use of donors <40 years (p=0.07) were correlated with improved OS. After allogeneic HSCT 75% (56/74) of the patients with PR and 65% (21/33) with RD achieved CR. The 5-year OS rates for patients with CR, PR and RD at the time of transplant were 64%/52%/39% (p=n.s.). Pretreatment with autologous HSCT had no impact on OS after allogeneic transplant.
Conclusion: Allogeneic HSCT in non-transformed FL is a feasible approach with a 100 d TRM of only 16%. 52% of the patients survived for more than 5 years with a minimal risk of late relapse. Even for patients with RD a substantial number of patients survived long-term.
This study was supported by the “Deutsche Krebshilfe e.V.”, Deutsche Jose-Carreras Leukämie Stiftung e.V. and DKMS e.V.

**P1124**

**Allogeneic haematopoietic stem cell transplantation in patients with transformed follicular lymphoma: data provided by the German Registry for Stem Cell Transplantation (DRST)**

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Background: Prognosis for transformed FL is poor with a median survival of <1 year with standard therapy. Autologous HSCT was associated with improved disease-free survival (DFS) and overall survival (OS) rates but high relapse rates remained a matter of concern. Presently it is unclear whether the use of allogeneic HSCT can improve clinical outcome or even cure transformed FL.
Patients and Methods: In the DRST database 34 patients with transformed FL were identified who underwent allogeneic HSCT between 1998 and 2007. 28/34 patients were pretreated with autologous HSCT. At time of allogeneic HSCT median age of patients was 51 years (range 28-59). 24/34 patients were either in complete (CR, n=3) or partial (PR, n=21) remission whereas 9 patients had refractory disease (RD). The conditioning regimen prior to allogeneic HSCT was reduced intensity in 25 and myeloablative in 9 of the 34 patients. All related donors (n=18) were HLA-identical siblings and of the unrelated donors (n=16) 69% were fully HLA-matched.
Results: All patients showed engraftment. 52% (11/21) of the patients with PR and 44% (4/9) of the patients with RD achieved a CR after allogeneic HSCT. For the whole study population, estimated 1, 2, 5-year DFS rates were 58%/46%33% and corresponding OS rates were 61%/49%/34%. Cumulative 100 d treatment-related mortality (TRM) was 26%. Post transplant, 4 patients relapsed (including one late relapse after 2.5 years) and 6 patients showed progressive disease. Best results after allogeneic HSCT were obtained for the subgroup of patients with controlled disease (CR/PR), use of RIC and matched donors (n=16) with a TRM of 13%, 1, 2, 5-year DFS rates of 71%/63%/52% and OS rates of 76%/68%/57%.
Conclusion: Allogeneic HSCT is a therapeutical option in patients with transformed FL. Best results were obtained for patients with controlled disease (CR/PR) prior to transplant, use of RIC and fully matched related/unrelated donors. However even 3/18 (17%) of the patients belonging to the high-risk group survived long-term.
This study was supported by the “Deutsche Krebshilfe e.V.”, Deutsche Jose-Carreras Leukämie Stiftung e.V. and DKMS e.V.

**P1125**

**Reduced-intensity allogeneic transplantation following autologous transplantation is feasible in patients with high-risk non-Hodgkin lymphoma**

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Introduction: Relapse after autologous stem cell transplantation (ASCT) remains the most important cause of failure among high-risk lymphomas. In order to improve outcome, allogeneic hematopoietic stem cell transplantation (AlloSCT) is used to exploit graft-versus-lymphoma (GVL) effect; on the other hand, feasibility of AlloSCT is limited by the high risk of infections and graft-versus-host disease (GvHD).
Patients and Methods: We retrospectively analyzed data on 34 high-risk NHL patients who underwent ASCT followed closely

by reduced-intensity AlloSCT ("tandem auto-allo") from January 2002 to November 2010. The tandem transplantation decision relied on the high-risk disease, needing some consolidation therapy after ASCT, and on the hypothesis of a high GVL effect when a significant cytoreduction occurred after AlloSCT. The search for an allogeneic donor was started at the beginning of salvage regimen. GvHD prophylaxis included cyclosporine alone or in combination with methotrexate. Only HLA-identical sibling or 10/10-matched unrelated were allowed. Results: median patients' age at AlloSCT was 47 (27-68). Main patient's and transplant characteristics are shown in Table 1. Peripheral stem cell was used in all patients but one. Median interval between ASCT and AlloSCT was 77 days (36-197).

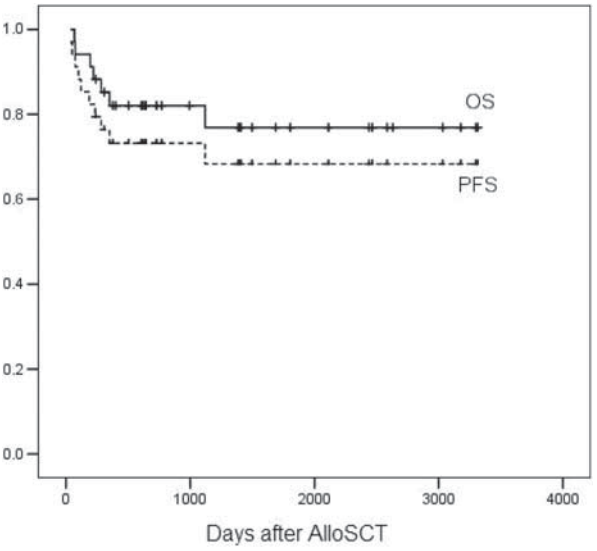
Successful allogeneic engraftment occurred in all patients. At a median follow-up of 46 (8-108) months since AlloSCT, 5-year overall survival (OS) is 77% (61-93) and progression-free survival (PFS) is 68% (51-85) (Figure 1). Disease relapse or progression occurred in six patients (cumulative incidence at 2 years=22%), 100-day treatment-related mortality (TRM) was 0%, 2-year estimate of cumulative incidence was 6%. Causes of TRM were sepsis with subsequent multiorgan failure in two patients and severe extensive chronic GvHD in one patient. Ten patients developed grade ≥2 acute GvHD and fifteen patients chronic GvHD, with a cumulative incidence of 29% (14-44) and 45% (27-63) respectively.

Conclusions: Fandem transplantation is feasible in high-risk NHL patients having a HLA-identical allogeneic stem cell donor, with 0% early TRM and 6% 2-year TRM incidence. This tandem approach could represent a suitable therapeutic option for those patients with high-risk NHL potentially benefitting from further therapy after ASCT, that is here GVL effect, after disease debulking by salvage therapy and ASCT. Donor search should promptly be started whether such an approach is chosen.

**Table 1.** Main patients' and transplant's characteristics

| | |
|---|---|
| Patients (n) | 34 |
| Gender (M/F) | 23/11 |
| Median age (range) | 47 (27-68) |
| Histology | |
| DLBCL | 5 |
| FL | 14 |
| Transformed FL | 4 |
| MCL | 5 |
| Plasmocytoid L | 1 |
| Anaplastic Large T cell lymphoma | 2 |
| Peripheral T cell lymphoma | 3 |
| Median prior therapeutic lines (range) | 2 (0-4) |
| Conditioning regimen for ASCT | |
| BEAM | 18 |
| Mel140 | 8 |
| Mel200 | 8 |
| Disease status pre-ASCT | |
| CR | 14 |
| PR | 14 |
| PD | 6 |
| Conditioning regimen for AlloSCT | |
| Fludarabine-busulfan-ATG | 25 |
| Fludarabine-cyclophosphamide ± thiotepa | 9 |
| Disease status pre-AlloSCT | |
| CR | 16 |
| PR | 13 |
| PD | 5 |
| Median days interval ASCT-AlloSCT (range) | 77 (36-197) |
| Donor (sibling/unrelated) | 29/5 |
| Risk factors | |
| Chemorefractory disease | 7 |
| Histology | 7 |
| Relapse after >= 1 line w/o prior ASCT | 9 |
| Relapse after prior ASCT | 4 |
| Unfavorable relapse | 7 |

**[P1125]**




**P1126**

## Induction of graft-versus-host disease by donor lymphocyte infusion and lenalidomide resulting in complete remission in a patient with hepatosplenic T-cell lymphoma relapsed after allogeneic stem cell transplantation

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Hepatosplenic T-cell lymphomas are rare but aggressive malignancies with a median overall survival time of less than two years. Conventional chemotherapy may initially lead to a complete remission, which, however, tends to be followed by relapse within less than a year. Currently the only promising approach to successful treatment appears to be allogeneic hematopoietic stem cell transplantation, which has been reported to improve 5 year overall survival rates to around 50%. In cases of relapse after allogeneic stem cell transplantation the prognosis has been considered utterly dismal.

We report here on a 25-year-old patient who presented with fever, nightsweats, fatigue and hepatosplenomegaly and was diagnosed with hepatosplenic t-cell-lymphoma with 30% bone marrow infiltration. Four blocks of chemotherapy did not manage to control the disease but after undergoing allogeneic stem cell transplantation from a matched related donor with TBI8Gy/fludarabine conditioning the patient achieved complete remission. However, a relapse occurred only four months later. Upon diagnosis of relapse a donor lymphocyte infusion (DLI) was given ($10x10^6$ CD3+ cells/kg), followed by two courses of lenalidomide (5 mg/d) one and two months later. Three months after the DLI the patient suffered a severe bout of Graft-versus-Host-Disease (GvHD) of the liver but also managed to achieve complete remission of the t-cell-lymphoma. An immunosuppressive therapy with corticosteroids, cyclosporine and mycophenolate was started, with which the GvHD of the liver was successfully controlled.

Under mycophenolate and tapering doses of cyclosporine the patient is now well and free of symptoms while still remaining in complete remission, one year after transplantation.

**P1127**

**The outcome of allogeneic stem cell transplantation in chronic lymphocytic leukaemia: 10-year experience of Polish transplant centres**

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Introduction: Chronic lymphocytic leukemia (CLL) is an incurable disease when treated with standard chemotherapy. The only possibility to provide cure is allogeneic stem cell transplantation (allo-SCT). CLL patients aged less than 55 account for about 15% of patients and these cases allo-SCT should be taken into consideration. The indications for allo-SCT are del17p, resistance to chemoimmunotherapy, Richter's syndrome or recurrent disease.

Patients and Methods: A retrospective analysis of allo-SCT in 31 patients (18 males, 13 females) with CLL transplanted in years 2000-2011 was performed. The aim of the study was to assess the long term follow-up outcome of allo-SCT in CLL patients.

Results: The median age at diagnosis was 43ys (range: 35-55). The sibling donor was available in 29 cases (2 pts were mismatched), unrelated donors were in 2 cases (1 mismatched). Median lymphocytosis before allo-SCT was 1.6 G/l. Peripheral blood was the source of stem cells in 21 cases (68%), and bone marrow in the remaining 10 cases, 2 pts were transplanted with stem cells from bone marrow and peripheral blood. 4 pts (13%) underwent the allograft procedure twice or more. Reduced intensity conditioning (RIC) was performed in 27 pts (13 of them – with alemtuzmab), myeloablative regimen in 4 cases and RIC with rituximab in one case. The median number of CD34+cellsx$10^6$/kg was 4.2 (range: 0.86-9.64). Four patients suffered from graft failure. Acute graft-versus host disease (GvHD) was noted in 45% of pts (only in 3 pts grade IV). Extensive GvHD was observed in 8 pts. Donor lymphocyte infusion (DLI) was performed in 11 pts (35,5%). With a median follow-up of 42 months (range: 1,8-129) for surviving patients, the five-year Kaplan-Meier of overall survival (OS) and progression free survival (PFS) was 48% and 34%, respectively. At five years, the cumulative probability of non-relapse mortality was 16%.

Conclusions: Allogeneic stem cell transplantation remains the only curative treatment in CLL and should be indicated for a selected group of patients.

**P1128**

**Allogeneic stem cell transplantation for patients with advanced primary cutaneous T-cell lymphoma**

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Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) provides a potentially curative option for patients affected by primary Cutaneous T-Cell Lymphoma (CTCL) in advanced stage. We are reporting 13 patients (8 F; 5 M) with a median age of 52 years (range 41-64 yrs) affected by advanced CTCL. Diagnosis were: panniculitis-like T-cell lymphoma (n=1) large T-cell lymphoma (n=3), NK T-cell lymphoma (n=1), Mycosis Fungoides (MF, n=2), Sezary Syndrome (SS, n=6). Seven patients had a relapsed/refractory disease, 4 patients were in partial remission and 2 in complete remission. All patients but 1 were heavily pretreated and 3 had undergone a previous autologous transplant. Six patients were transplanted from a HLA identical sibling, 1 from a HLA mismatched sibling, 4 patients were transplanted from a matched unrelated donor and 2 from umbilical cord blood (UCB) unit. Nine patients received a RIC regimen consisted of thiotepa, fludarabine and cyclophosphamide (n=7) and thiotepa, fludarabine and busulfan (n=2). Four patients received a myeloablative conditioning (MAC) consisted of cyclophosphamide and busulfan in 2 patients and thiotepa and fludarabine and busulfan in 2. Cyclosporine and methotrexate were used as GVHD prophylaxis in HLA identical transplant. UCB transplant received cyclosporine and mycophenolate mofetil and cyclosporine and prednisone, respectively. Thymoglobulin was added in mismatched transplant. Neutrophil and platelet engraftment were achieved in all but one patients. A grade I-II acute GVHD developed in 4 patients, grade III-IV in one. Chronic GVHD (cGVHD) was observed in 11 patients: 9 limited and two extended. Eleven patients were evaluable for response (2 too early) and achieved a complete remission. Five patients experienced relapse at 3, 8, 9, 10, 17 months from HSCT. Three of them infused donor lymphocyte: a second durable remission was obtained in 2 SS patients, no response was observed in patient with NK T lymphoma. Transplant-related mortality occurred in four patients (3 MAC regimen, 1 RIC regimen). They died as result of multiorgan failure (n=3) and infection plus extensive cGVHD (n=1), 2 at 3 months, 1 at 7 months, 1 at 11. To date at median follow up of 45 months (range 20-85) 9 patients are alive, 6 without evidence of lymphoma. Our results show a lower transplant-related toxicity by using RIC regimen, suggest a potential curative effect of allogeneic RIC transplant and indicate the existence of a graft versus tumor effect in CTCL.

**P1129**

**Allogeneic stem cell transplantation for relapsed/refractory lymphoma after conditioning with BEAM/Fludarabine/TBI – an analysis of 50 consecutive patients**

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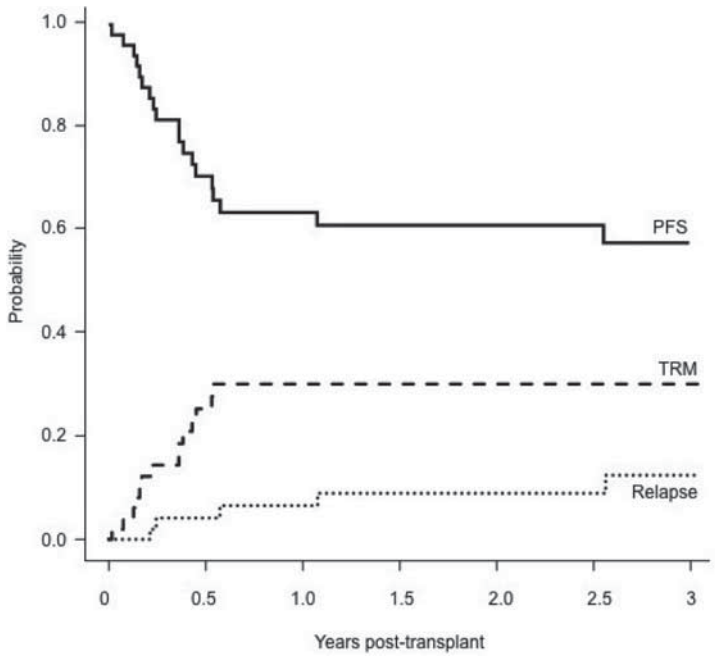
Background: Autologous stem cell transplantation (HSCT) is the standard of care for relapsed/refractory lymphoma. While auto-HSCT is not curative in the majority of cases, allogeneic HSCT can provide a further rescue option. Previously, we reported on our experience of allo-HSCT after conditioning with BEAM/Fludarabine/total body irradiation (TBI) in 10 patients with relapsed/refractory lymphoma, an approach that combines the cytoreductive properties of BEAM with the graft-versus-tumor effect of transplantation. After treating 50 consecutive patients with this regimen, we now provide a final analysis.

Patients and Methods: Between 2002 and 2011, 28 male and 22 female patients age 22 to 64 (median 49) were included. All patients had refractory or relapsed chronic lymphocytic leukemia (13 patients), Hodgkin's lymphoma (8) or Non-Hodgkin lymphoma (29). Patients had undergone a median of 5 previous treatment lines (range 1-8), including auto-HSCT in 44%. Disease status at transplant was progressive in 8 (16%), stable disease in 8, partial remission in 21 (42%) and complete remission in 13 (26%). Donors were HLA-identical siblings (58%), 10/10 matched unrelated (38%) or 9/10 matched unrelated (4%). The conditioning regimen consisted of 3x25 mg/$m^2$ Fludarabine administered overlapping standard BEAM chemotherapy, followed by a single dose of TBI (2Gy) on the day of transplantation. Graft-versus-host disease (GvHD) prophylaxis consisted of cyclosporine and a short course of mycophenolate mofetil.

Results: With a median follow-up of 3.4 years, 30 patients (60%) are alive. All patients achieved engraftment and full donor chimerism. The major transplant-associated toxicity was mucositis (WHO grade 4 in 62%, 3 in 30% and 2 in 8%). Acute GvHD grade 2-4 arose in 64% and chronic GvHD in 51% of patients. At three years, Kaplan-Meier estimated progression free survival was 58%, with 30% transplant-related mortality

[P1129]




and a relapse incidence of 12% (Figure). Causes of death were GvHD (n=10), infection (n=4), cerebral haemorrhage (n=1) and relapse (n=5). Disease classification, stage at transplant and type of previous treatment all had no significant impact on transplant outcome.
Conclusion: Allo-HSCT after BEAM/Fludarabine/TBI provides excellent disease control in patients with advanced lymphoma. High rates of GvHD and GvHD-related mortality associated with this regimen are a major concern and may warrant modification of the regimen in the future.

**P1130**

**Unrelated reduced-intensity cord blood transplantation for relapsed/refractory aggressive B-cell non-Hodgkin lymphoma**

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The status of unrelated cord blood transplantation (uCBT) for relapsed/refractory aggressive B-cell non-Hodgkin lymphoma (B-NHL) has not been determined yet.
We retrospectively analyzed 31 patients who received uCBT for relapsed/refractory aggressive B-NHL from January 2005 to Jun 2011 in Toranomon Hospital. In this analysis, the patients with 2nd allogeneic transplant, PS 3-4, and active infectious diseases at transplant were excluded. Nineteen (61.3%) were male and the median age at uCBT was 51 y.o. (20-66). Eighteen patients (58.1%) were 50 y.o. or older. Concerning histological diagnosis, 25 were DLBCL-NOS, 4 were transformed lymphoma, and 2 were Burkitt lymphoma. Eleven patients (35.5%) had chemosensitive disease and disease status was CR in only one case. Twelve (38.7%) had previous history of autologous transplantation and 15 (48.4%) had received prior >3 regimens. LDH level at transplant was more than upper normal limits in 25 (80.6%) cases. Reduced-intensity conditioning (RIC) regimens were used in all but 3 cases. All cases were transplanted with single cord blood unit.
Two-year progression-free survival (PFS) and overall survival (OS) were 12.2% and 30.6%, respectively. The median follow-up period of survivors was 23 months (1-75). The rates of non-relapse mortality (NRM) and disease progression at 2 years after uCBT were 29.7% and 58.1%, respectively. The number of regimens before uCBT (>3 regimens) and grade 2-4 acute GvHD were poor prognostic factors for OS. No factors were attributed to PFS. Time from diagnosis to uCBT (> 1 year) and grade 3-4 acute GvHD were risk factors for NRM, and non-TBI regimen was a risk factor for disease progression. Two-year PFS and OS were higher in chemosensitive group (PFS: CR/PR 12.7% *vs.* SD/PD 6.6%, OS: CR/PR 40% *vs.* SD/PD 25%), but not significantly different.
Because long-term suivival can be expected in spite of high disease-progression rate, unrelated RIC-CBT can be a option for treatment of relapsed/refractory aggressive B-NHL. We should consider another therapeutic strategies, such as timing of transplantation, choice of autologous or allogeneic, strength of conditioning regimen, and maintainance therapy after uCBT.

**P1131**

**Rituximab-bendamustine prior to donor lymphocyte infusion as salvage treatment in Hodgkin's disease patients relapsed post allogeneic stem cell transplantation**

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Background: Hodgkin Disease (HD) patients (pts) relapsed after allogeneic Stem Cell Transplantation (allo-SCT) have a poor prognosis. Salvage strategies are immunosuppression withdrawal or Donor Lymphocyte Infusions (DLI); chemotherapy (CT) with the purpose of tumor debulking and lymphodepletion is an attractive option prior to DLI. We investigated the role of Bendamustine (Benda), an alkylating agent which has proved to be highly effective in salvage treatment of lymphoproliferative disorders and HD.
Aim: To evaluate the feasibility of Rituximab-Bendamustine (R-Benda) prior to DLI as a salvage therapy in pts with HD relapsed after allo-SCT.

Patients and Methods: Between 06/2009 and 10/2010, 5 pts with HD (stage II-IV) who underwent allo-SCT (1 MUD, 4 HAPLO) after a reduced toxicity conditioning Treosulfan-based and relapsed at a median time of 262 days after transplant (range 186-397). Salvage treatment with Rituximab 375 mg/mq on day 1 and Benda on day 2-3 every 28 days was planned. Benda, always preceded by adequate premedication, was administered at the dose of 120 mg/mq/day in 3/5 pts, 90 mg/mq/day in 1/5 pts and 70 mg/mq/day in 1/5 pts. CT cycles and concomitant supportive treatments were administered in outpatient regimen. To assess treatment related toxicities weekly blood examinations and clinical monitoring were performed.
Results: a median of 4 cycles of R-Benda (range 1-6) was administered. One patient progressed prior to the first re-evaluation and died rapidly from HD. In 4 evaluable pts, we observed 1 case of grade IV thrombocytopenia and 3 cases of grade IV neutropenia, but no severe infectious events were reported. In 1 case the induced cytopenia required a Benda dose reduction (from 120 mg/mq/day to 90 mg/mq/day) and concomitant treatment delay. Only one case of grade II gastrointestinal toxicity (nausea) and 1 case of cutaneous rash following Benda infusion were observed. No admission was necessary. To date, 2 pts died from disease progression, and 3 are alive after a median follow up of 155 days (range 101-328); 3/3 achieved a partial response at the last TC/PET disease evaluation.
Conclusions: R-Benda salvage regimen has a good tolerability profile with manageable hematological toxicities and no severe extra-hematological complications in pts with relapsed HD after allo-SCT. A larger series of pts is needed to validate these data and assess the efficacy of Benda in treating HD relapsed post allo-SCT.

**P1132**
**Serious transplant-related toxicity in patients with Hodgkin's disease previously treated with brentuximab vedotin – a case series**
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Background: Allogeneic hematopoietic stem-cell transplantation (HSCT) is an established treatment for refractory/relapsed Hodgkin's disease (HD). Treatment with brentuximab vedotin (BV) has recently shown encouraging results in refractory HD. However, there is little experience with potential interactions of treatment with BV and subsequent HSCT.
Methods: We report on 3 male Caucasian patients in whom treatment with BV made allogeneic HSCT possible. Patients received conditioning with BEAM combined with fludarabine and 2Gy TBI which has been previously described.
Case series:
Patient 1, 26y, was diagnosed with nodular slerosing HD in 08/09 and treated with 4 lines of therapy including autologous HSCT. He received 4 cycles of BV for massively progressive lung disease achieving PR, and received an allogeneic HSCT in 06/11. Posttransplant complications included acute graft-versus-host disease (aGvHD) grade III (cutaneous and intestinal), and haemorrhagic cystitis. He was discharged on day +33 and later rehospitalized for CMV oesophagitis and pneumonia, which resolved.
Patient 2, 25y, was diagnosed with lymphocyte depleted HD in 03/09. He failed 7 lines of therapy before receiving 10 cycles of BV achieving a PR. After autologous HSCT he received an allogeneic HSCT in 09/11. He subsequently developed mucositis and alveolar haemorrhage, but no GvHD. He was discharged on day +23 and rehospitalized for bilateral pneumonia on day +54. He developed an EBV-associated haemophagocytic syndrome, required mechanical ventilation and died on day +69 due to acute respiratory distress syndrome.
Patient 3, 32y, diagnosed with nodular slerosing HD in 05/09, was treated with 3 lines of therapy and autologous HSCT with early relapse, received 10 cycles of BV with a transient response and subsequent progression. In 09/11 he received an allogeneic HSCT. He developed transplant-associated microangiopathy and aGvHD grade III (cutaneous and intestinal) with pneumatosis intestinalis. 78 days after HSCT, he remains in hospital with GvHD.
Conclusion: All 3 patients who received BV before allogeneic HSCT showed important toxicity and severe complications. It is unclear whether this is due to previous treatment with BV, multiple previous therapies, or both. Patients with refractory HD have a poor prognosis and chemosensitivity is usually a prerequisite for HSCT. It remains to be shown whether response to BV may benefit previously refractory patients longterm if combined with allogeneic HSCT.

**P1133**
**Allografting in relapsed/refractory Hodgkin's lymphoma, a single-centre experience**
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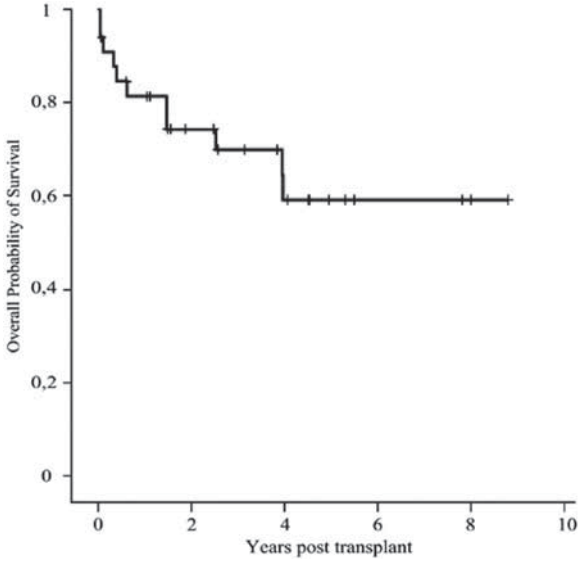
Hodgkin lymphoma (HL) can be cured in more than 90% of early-stage and about 75% of advanced-stage patients with conventional chemotherapy. Thus, only a minority of patients became candidate for allogeneic stem cell transplant (allo-SCT), however it remains the only curative option for refractory HL. We report a retrospective analysis of 33 relapsed or refractory HL patients, median age 33 (range 18-55) years, treated with reduced intensity (n 26/33, 79%) or myeloablative (n 7/33, 21%) allo-SCT between May 2000 and December 2010. Donors were HLA identical siblings (n 13/33, 39%) or matched unrelated (n 20/33, 61%). Twenty-nine/33 (88%) patients received more than 2 lines of treatment before allo-SCT, and 8/33 (24%) patients had chemorefractory disease at the time of transplant. Thirty-one/33 (94%) patients received at least one autologous-SCT, and 23/33 (70%) were treated with radiotherapy before allo-SCT. The cumulative incidence of non-relapse mortality (NRM) was 12,3% at 12 months from transplant. All NRM events occurred in patients treated with reduced intensity conditioning. The cumulative incidence of any grades acute graft-vs.-host disease (GvHD) was 43% at day 100, whereas

**[P1132]**

| | Donor | GvHD prophylaxis | Mucositis | aGvHD | Severe complications |
|---|---|---|---|---|---|
| Patient 1 | Unrelated, 10/10 match | ATG/CSA/MMF | Grade 4 | 3 | Haemorrhagic cystitis CMV oesophagitis |
| Patient 2 | Unrelated, 9/10 match | ATG/CSA/MMF | Grade 3 | None | Diffuse alveolar haemorrhage, bilateral pneumonia, ARDS |
| Patient 3 | Unrelated, 10/10 match | CSA/MMF | Grade 3 | 4 | Transplant associated microangiopathy, diffuse intravascular coagulation |

that of overall chronic GvHD was 52% at 400 days. After a median follow-up of 48 months (range 1-106), median overall survival (OS) and event-free survival (EFS) were respectively not reached and 23 months. Among patients with OS longer than 46 months (n=12, 36%), 8/12 (67%) were disease-free. With the limitation of a small sample size no difference in terms of OS and EFS were detected between the conventional and reduced intensity conditionings. Allo-SCT is a feasible option in heavily pre-treated relapsed or refractory HL patients with low NRM, and can induce durable clinical remissions. Furthermore, this study suggests graft-vs-HL effect exists and gives rise to long term disease control.




**P1134**

**AHSCT preceded by IVE and BEAM as effective treatment in HD patients who didn't achieved CR: a single-centre experience**

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Hodgkin lymphoma (HD) is one of the frequently and potentially curable haematological neoplasm, most patients (pts) are curable with chemo- and radiotherapy but the rests achieve only partial remission or have relapse after treatment.
In Department of Hematology and BMT Medical University of Silesia, between January and November, 2011, 10 pts with refractory or relapsed HD-CR was not reached at least after two lines of therapies (8 male, 2 female, median age 34 (19-56 years) - were undergone high dose myeloablative chemotherapy with BeEAM. Ann Arbor stage was as follow: II-3 patients, III-3 patient, IV-3 pts; 50% presented B-symptoms. The primary involved sites were: lymph nodes (n=10), bone marrow (n=1), lung (n=1). Every patients achieved a partial remission (PR) defined as the reduction of measurable disease by ≥50% without the appearance of any new lesions after ad least three lines of therapy (ABVD, BEACOPP, ESHAP, radiotherapy). Then pts were qualified to autologous hematopietic cell transplantation (autoHCT) using conditioning: Bendamustine 160 mg/m$^2$ iv in -8-7d, Etoposide 200 mg/m$^2$ iv in-6-3 d, AraC 600 mg/m$^2$ iv in -6-3d, Melphalan 140 mg/m$^2$ iv in -2d. Mobilization and transplantations procedures were performed during hospitalization. Peripheral blood stem cells were mobilized after IVE (Ifosamid 3 g/m$^2$ iv in 1-3d, Etopiside 200 mg/m$^2$ iv in 1-3d, Epirubicine 50 mg/m$^2$ in 1d) and G-CSF (10 ug/kg/d, from +5 day after chemotherapy till the last day of mobilization). All pts collected sufficient for autoHCT the number of CD34+ cells. The median number of transplanted CD34+ cells was 3,87 (2,1 -11,3x10$^6$/kg b.w.). All patients engrafted. Hematopoietic recovery was as followed: WBC count above 1,0 G/l 12days (9-15) neutrophil count above 0,5 G/l 11days (9-14) platelet count above 20 G/l 11days (9-23). Early complications after AutoHSCT were mainly: infection (fungal oral infection -1, mucositis -8). All patients are alive with the median observation time 111 (43-243 days). Two of them early relapse after few months. One of them had sibling donor and was underwent successfully allotransplantation, the second was qualified to anty CD30+ treatment.
Conclusions: High dose chemotherapy [IVE as mobilization and BeEAM as conditioning regimens] with AutoHSCT support is effective and non toxic regimens in HD patients, who did not achieved CR after standard treatment.

**P1135**

**High-dose chemotherapy and autologous stem cell transplantation for primary refractory or relapsed Hodgkin's and non-Hodgkin lymphoma: monocentre study and long-term outcome in 330 patients**

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Objective: To analyze clinical outcome and significant prognostic factors for overall survival (OS) and progression free survival (PFS) in patients(pts) with Hodgkin (HD) and non Hodgkin lymphoma (NHL) undergoing autologous stem-cell transplantation (ASCT).
Methods: Between October, 1987 and November, 2010 330 pts with HL and NHL that were not controlled by conventional chemotherapy underwent high-dose chemotherapy and ASCT by our transplant centre.
Results: 216 pts were NHL and 114 HD, 183 were male, median age was 39 years (range, 15 to 67 years). At the time of ASCT 161 (125 NHL, 36 HDG) were in complete remission (CR), 72 (55 NHL, 17 HDG) were in partial remission (PR) and 97 (36 NHL, 61 HDG) presented a resistant disease (RD). All pts received chemotherapy with high-dose regimens: 126 were treated with BEAM, 119 with ICE, 67 with Mitoxantrone-Melphalan and 18 were treated with other type of therapy. To proceed to ASCT, pts had to have adequate cardiac, pulmonary, hepatic, and renal function. The median time to engrafment was 11 (range 7-20) days for absolute neutrophil count (>500), 11 (range 5-40) days for platelets (20.000). After ASCT 204 pts were in CR, 44 were in PR and 82 presented a RD. With a median period of observation of 61 months (CI 95% 54-67) 221 pts are alive and 109 are death, the overall survival (OS) calculated with the Kaplan Mayer method is 59%. With a median period of observation of 49 months (CI 95% 40-52) 162 pts are free from progression and the progression free survival (PFS) is 49%. No differences in OS or PFS were observed between NHL and HD (respectively 67% *vs* 55% and 51% *vs* 46%). In NHL OS was significantly superior in pts arrived at transplant in CR (p:.003), and in pts treated with no more than two previous chemotherapy (p:.002); in HD, OS was affected only by the CR status at transplant (p:.01). Similarly, in NHL PFS was significantly affected by CR status at transplant (p:.0000) and to have received less than three previous therapy (p:.001); in HD only CR status at transplant affected significantly the PFS (p:.0004).
In conclusion this monocentric data confirm the importance of ASCT as salvage treatment either in NHL or in HD, in NHL it seems better to use this strategy earlier in the history of the disease while in HD even pts treated with several therapy, reaching a response before transplant, obtain excellent results. Finally chemo-sensibility remains the most important factor for prognosis.

**P1136**

**Autologous stem cell transplantation for relapsed/refractory Hodgkin's lymphoma: a single-center experience**

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Nowadays around 80% of patients with Morbus Hodgkin can be cured and the prognosis for this subset of patients has substantially improved. This goal was achieved with introduction of effective combination of chemotherapy regimens, progress in radiation techniques, and constant optimization of treatment methods. Although, Hodgkin lymphoma is considered a curable disease in approximately 75% of the cases, approximately 50% of patients relapsing after the first-line chemotherapy can be rescued by an autologous stem cell transplantation, whereas the others, which account for approximately 10-15% of the overall population, have a very poor prognosis. Randomized trials support the use of high dose chemotherapy and autologous stem cell transplanatation in patients with relapsed Hodgkin lymphoma, having shown that this management improved disease-free survival. We retrospectively analyzed efficacy and prognostic factors in 29 patients (20M/9F) with chemosensitive/chemoresistant relapse or refractory disease who underwent high dose chemotherapy followed by autologous stem cell transplantation from 2000-2010 in our institution. All patients were initially treated with ABVD regiment as first-line treatment. DHAP regimen was used as salvage protocol for relapsed/refractory patients. Patients have been harvested during salvage regiment. BEAM was the main conditioning regiment. There was no treatment related deaths. The five years overall survival rate was 53%. The presence of active disease at transplant, and two or more lines of therapy before transplantation were adverse prognostic factors for outcome. Significant prognostic factor that influence the overall survival is disease status before transplant. Overall survival was significantly better in patients with in first relapse (67%) than in patients with primary refractory -induction failure and advanced disease (25%). High-dose therapy and autologous stem cell transplantation seems to be highly effective and safe procedure in patient with relapsed/refractory Hodgkin lymphoma. There is still room for improvement in the subset of patients relapsing after autologous transplant or patients with chemoresistant disease.

**P1137**

**High-dose chemotherapy and autologous stem cell transplantation for Korean patients with relapsed or refractory Hodgkin's lymphoma**

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Background: High-dose chemotherapy and autologous stem-cell transplantation (ASCT) is a standard therapy for patients with relapsed or refractory Hodgkin lymphoma (HL). However, its efficacy in Asian patients with relapsed or refractory HL has not been studied well perhaps because of its low incidence in Asia.
Patients and Methods: A retrospective analysis of outcomes in 10 consecutive patients who underwent ASCT for HL in a single center from August 2005 to September 2010 was conducted.
Results: The median age was 34.5 years old (17-64) and 7 patients were male. Four patients were in stage I-II and the others were in stage III-IV at presentation using Ann Arbor stage. B symptoms were present in four patients. Four of the patients had International Prognostic Score ≥ 3 at diagnosis. The analysis included nine relapsed HL and one primary refractory case. Four patients were in second complete remission (CR) and others were in partial response after salvage chemotherapy. All the four patients in CR before ASCT continued to be in CR and four of six patients who were in PR achieved CR after ASCT. Three year progression free survival (PFS) and overall survival (OS) from ASCT were 50% and 76% with a median follow-up duration of 41.2 months. CR at time of ASCT seemed to be associated with better PFS despite lack of statistical significance due to small sample size (HR 0.223, 95% confidence interval 0.024-2.050, p=0.185).
Conclusions: Our results suggest that the efficacy of high-dose chemotherapy with ASCT in Korean patients with refractory or relapsed HL may be comparable with previous reports from mainly Caucasian patients.

**P1138**

**Autologous haematopoietic stem cell transplant in patients with Hodgkin's lymphoma. A single-centre experience**

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Introduction: The prognosis of patients with Hodgkin lymphoma (HL) primarily refractory or who relapse worsens considerably. Treatment usually includes high-dose chemotherapy with hematopoietic stem cell transplant (Auto-HSCT) rescue with good results. We present our experience in HL and Auto-HSCT.
Objectives: To analyze retrospectively the response to auto-HSCT in patients with HL The variables included are: pre auto-HSCT state, response to auto-HSCT, overall survival and median survival, treatment-related mortality and cause of mortality.
Materials and Methods: During the period from May 98 to November 2011, 25 patients diagnosed of HL underwent auto-HSCT. One patient received a double auto-HSCT. The treatment at diagnosis was ABVD (n=18), COPPABV (n=3) and MOPPABV (n=2). Patients elegible for Auto-HSCT were: a) primary refractory patients (didn't reach complete response (CR) after initial treatment, progress during treatment, duration of CR <2 months) (n=10) and b) patients with late relapse (n=15). The conditioning treatment used was BEAC (n=20), BEAM (n=3), ICE (n=1) and CBV (n=1). In 2 cases the response was consolidated with radiotherapy. Post auto-HSCT reevaluations were performed by image studies on day + 100, then every 3 months and after 2 years every 4 months.
Results: The response to auto-HSCT on day + 100 was: CR (n=18), RP (n=4), progression or stable disease (n=3). The median survival was 47.1 months from auto-HSCT with an overall survival of 64%. The transplant related mortality (before day + 100) was n=1 (4%) for pulmonary toxicity. The cause of death of the other 8 patients were due to progression (n=3), malignancies (n=3) and cardiovascular complications (n=2). Of the survivors, 1 developed high-risk MDS, and one rectal cancer.
Conclusions: 1. - Auto-HSCT rescue therapy is effective in our environment with a low procedure-related mortality. 2. - In our short series, there seems to be no difference in overall survival between refractory transplant patients and those with late relapse.

**P1139**

**Brentuximab vedotin (SGN-35) in Hodgkin's lymphoma patients relapsed after autologous peripheral blood stem-cell transplantation**

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Background: Approximately 15 to 30% of patients with Hodgkin's lymphoma (HL) do not have a long-term remission with conventional therapy. Autologous peripheral blood stem-cell

transplantation (APBSCT) represents a potentially curative treatment for some patients with recurrent or progressive HL after failure of initial combination chemotherapy. Unfortunately, APBSCT is only effective in approximately 50% of such patients. Brentuximab vedotin comprises an anti-CD30 antibody conjugated by a plasma-stable linker to the potent antimicrotubule agent, monomethyl auristatin. Brentuximab vedotin selectively induces apoptotic death of CD30+ cells.
Methods: We evaluated the efficacy and safety of brentuximab vedotin in patients (pts) with relapsed HL after APBSCT. Pts received brentuximab vedotin 1.8 mg/kg q3 weeks (wks) as a 30 minute outpatient IV infusion for up to 10 cycles. The primary endpoint was the objective response rate (ORR) and toxicity.
Results: Five pts were enrolled; four pts were male and median age was 25 yrs (range, 18-28 yrs). Pts had received a median of 4 (range 3-5) prior cancer-related systemic therapies excluding APBSCT. Eighty percent of pts had primary refractory disease and 80% had not responded to their most recent prior therapy. Tumor regression occurred in 80% of patients and the overall objective response rate was 40% (n=2), with partial remissions (PRs) in 2 pts. The most common treatment-related adverse events (AEs) of any grade were alopecia, abdominal pain, fatigue, insomnia and diarrhea. AEs ≥ grade 3 occurred in ≥ 60% of pts were fatigue, insomnia artralgia and diarrhea.
Conclusions: With manageable AEs, single-agent brentuximab vedotin induced objective responses in 40% of pts with relapsed HL after APBSCT. In this heavily pretreated population, 2 of 5 pts (40%) achieved PR and tumor regression and clinical improvement occurred in 80% of patients.

**P1140**
**High-dose etoposide, cytarabine and melphalan as conditioning regimen for autologous stem cell transplantation in patients with refractory or relapsed Hodgkin's lymphoma**
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Objectives: The efficacy of high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) for refractory or relapsed Hodgkin's lymphoma (HL) has been reported, but an optimal conditioning regimen has not been determined. Several chemotherapy preparative regimens are used, however, there has not been a randomized clinical trial to support the superiority of one regimen over another. We report treatment results achieved for refractory or relapsed HL in adult patients with a new conditioning regimen.
Patients and Methods: From March to September 2011, all patients with HL were treated with high-dose Etoposide (800 mg/m²), Aracytine (8 g/m²) and Melphalan (140 mg/m²) followed by reinfusion of peripheral ASCT (EAM).
Results: A total of 14 patients were retrospectively evaluated. The median age was 27 years (range; 17-47), median number of CD34+ cells was $3{,}86x10^6$/l (2,71–6,15). All patients had a full haematopoietic reconstitution. Median time to achieve neutrophils >500/µl was 13 days (range;10-19) and median time to achieve an unsupported platelet count >20000/µl was16 days (range; 14-25).Toxicities included grade 4 hematologic in 14/14 patients, grade 3 mucositis in 4, grade 3 infectious in 2. No case of transplant-related mortality occurred. After a median follow up of 6 months, all patients are alive and all are in continuous CR.
In conclusion, the EAM regimen would be an effective and tolerable conditioning regimen with acceptable engraftment and toxicity for ASCT for refractory or relapsed HL. Although these outcomes are encouraging, longer follow-up is required and comparison with other traditional ASCT regimens used for patients with refractory or relapsed HL is warranted.

**P1141**
**Impact of pre-transplant rituximab and PET on clinical outcome after autologous stem cell transplantation for diffuse large B-cell lymphoma**
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Background: Salvage chemotherapy (CHT) followed by high-dose therapy (HDT) and autologous stem-cell transplantation (ASCT) is the standard treatment for relapsed diffuse large B-cell lymphoma (DLBCL). Rituximab (R) improves outcome of salvage CHT in R naive pts. However, the impact of R-salvage CHT in pts who have failed after R-containing induction remains questionable.
Aims: We retrospectively analysed the impact of addition of rituximab to CHT followed by HDT and ASCT and the role of PET in staging of DLBCL.
Methods: There were 219 pts with DLBCL who underwent ASCT at our institution between 1994-2010. The median age at the time of ASCT was 45 years (18-70). Median follow-up was 4.9 y (0.1 -9.6) in R-CHT group (152 pts) and 13.2 y (0.1-17.3) in CHT group (67pts). 81 pts underwent HDT with ASCT as a part of intensive induction therapy, 60 pts as a consolidation after standard induction treatment and 78 pts for relapse or progression (R/P). There were 3 groups of pts in R/P cohort: 22 pts received R in induction as well as in salvage therapy, 24 pts were treated with R only in salvage therapy and 35 pts never received R.
Results: At 5 years from the date of ASCT, the estimated overall survival (OS) was 79% in R-CHT group *vs* 57% in CHT group (p=0.001) and the progression-free survival (PFS) was 72% *vs* 51% (p=0.001). A significant difference in OS and PFS was observed in favor of R-CHT group in pts who underwent HDT with ASCT as a part of induction therapy or consolidation: OS 83% *vs* 64%, (p=0.02), PFS 77% *vs* 58%, (p=0.03) in 5y. In R/P cohort, pts who received R-CHT as a salvage treatment without prior exposure to R had significantly better outcome compared to R-naive pts: OS 79% *vs* 48% (p=0,03), PFS 70% *vs* 41% at 5y (p=0,03). There was no significant difference compared to pts who were treated with R in induction as well as in R/P: OS and PFS were 56%, resp. 47% (p=0,09, resp. p=0,12) for this group of pts. Pts with negative pretransplant PET had significantly better outcome compared to PET positive pts: OS 84% *vs* 58% (p=0,001), PFS 75% *vs* 58% (p=0,02) in 5y. Positive predictive value (PPV) of pretransplant PET was 44%, negative predictive value (NPV) was 81%. After ASCT, PPV of PET was 66%, NPV 88%.
Conclusion: R significantly improved OS and PFS in pts transplanted in induction treatment as well as in pts who have failed after induction therapy without R. Pts who have failed after R-containing induction had a comparable benefit from ASCT as pts who were R-naive. NPV of PET is high prior as well as after ASCT, PPV of PET increased after ASCT.

**P1142**
**FDG PET-based response assessment before high-dose chemotherapy with peripheral blood stem cell and outcome, in patients with diffuse large B-cell lymphoma**
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Background: High dose chemotherapy (HDC) with peripheral blood stem cell (PBSC) is the treatment of choice for diffuse large B cell lymphoma (DLBCL) relapsed/progressed after first line chemotherapy (CT). This treatment is applied also for patients with high risk IPI as frontline therapy It is usual to consider eligible, for this kind of treatment, patients reaching complete remission (CR) or partial remission (PR) after CT. In many

studies, outcome is not different for CT scan-defined CR or PR. FDG PET is now the most sensible technique to evaluate the response to treatment. Actually, it is not so clear if patients with FDG PET CR and PR have the same outcome after HDC.
The aim of this retrospective study was to analyse if FDG PET-based CR and PR carry out a different outcome after HDC and PBSC.
Patients and Methods: Starting from 2002, DLBCL patients relapsed/progressed after first line CT, received the same salvage CT consisting of vinorelbine, ifosfamide and high dose cytarabine (VIHA), associated to rituximab (n= 30). After 4 cycles, response was evaluated by FDG PET. Patients with CR or PR underwent to HDC with PBSC harvested after the 3rd or 4th VIHA. Patients with IPI 2-3 DLBCL was also included (n= 36). Conditioning regimen consisted of BEAM. Progression free survival (PFS) and overall survival (OS) were calculated from the day of PBSC infusion.
Results: 67 patients were included and the median follow up was 38 months (range 1-100). Of these, 43 (64%) were in CR, 16 (24%) in PR, and 8 (12%) in PD, at time of HDC. The median age was 55 (range 23-74). For all patients, the median PFS was 43 months (range 0.9-100) and the median OS was not reached. The 3-year PFS and OS were 61% and 73%, respectively. The median PFS was 58 months, 37 months, and 5 months, for CR, PR, and PD patients (p <0.001). Considering patients in CR and PR, the median PFS was 58 and 37 months, respectively, and the 3-year PFS was 68% and 65, respectively (p 0.5).
Eighteen patients dead, and most of them because of lymphoma progression (67%). The non relapse mortality rate was 4%. Late events were recorded in 12 patients (8%).
Conclusions. This retrospective study showed that the outcome of DLBCL patients was not different if CR or PR FDG PET-defined was obtained before HDC.

**P1143**
**High-dose melphalan with autologous stem cell support in FDG PET-refractory lymphoma patients is effective and safe therapy as bridge to second transplant**
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Background: Disease status before high dose chemotherapy (HDC) is the strongest factor predicting the outcome of lymphoma patients (LP). Patients obtaining a complete (CR)or partial remission (PR) after salvage chemotherapy (CT) are considered chemosensitive and are allocated to HDC with survival estimated to be almost 50%. FDG PET is now the standard to evaluate disease response, and the prognosis of LP with PR seems to be worse than those in CR.
The treatment of LP not reaching CR after a first salvage CT with conventional salvage regimens does not seem effective.
We previously published the efficacy of HD melphalan (PAM) in 42 patients with active disease, without FDG PET evaluation. In this analysis were included only the patients with active disease after salvage CT, evaluated by FDG PET. These patients were all candidates to tandem auto-auto or auto-allo program, based on donor availability. The aims were to establish the activity of HD PAM in the FDG PET-era and to define the probability to proceed towards the therapeutic program.
Patients and Methods: From 2002, 44 LP with active disease, defined as less than PET-defined CR after the last salvage CT, were analysed. Main patient characteristics are reported in the table. Forty patients were evaluable because disease response was evaluated by FDG PET. Results were reported as intention to treat (ITT) and per treatment.
Results: The median time between HD PAM and FGD PET evaluation was 31 days (range 14-54). The overall response rate (ORR) was 70% and 77%, in ITT and per treatment analysis. In ITT, CR and PR rate were 38% and 31%, respectively. ORR to HD PAM was analysed based on the disease status before CT (PR *vs* SD/PD), and not significative difference was observed (ORR PR-group 80% *vs* ORR SD/PD group 73%). The established therapeutic program was maintained in 70% of patients scheduled for an auto-auto and 73% for those scheduled for auto-allo.
The median time to absolute neutrophil count above 0.5/mm$^3$ was 11 days (range 9-17). Infectious complications were mild and one patient developed non severe neutropenic enterocolitis. The incidence of severe (G3-4) oral mucositis was 50%. No toxic deaths were observed after HD PAM.
Conclusions: This retrospective analysis showed that: 1) HD PAM is effective in LP with FDG PET active disease, and the ORR seems higher than reported in the literature using conventional salvage CT (almost 30%); 2) in two third of patients, HD PAM is an effective bridge to second transplant.

| | N 44 |
|---|---|
| Median age (range) | 33 (16-61) |
| Sex M/F | 26/18 |
| Histology | |
| NHL | 16 |
| HL | 28 |
| Disease response before HD PAM | |
| PR | 25 (63%) |
| SD/PD | 15 (27%) |
| Therapeutic program | |
| Auto-auto | 29 |
| Auto-allo | 15 |
| N CD34/kg infused | 4.5 (1.7-15) |
| | |

**P1144**
**Prognostic value of pre-transplant positron emission tomography using fluorine 18-fluorodeoxyglucose in patients with lymphoma treated with high-dose chemotherapy and stem cell transplantation**
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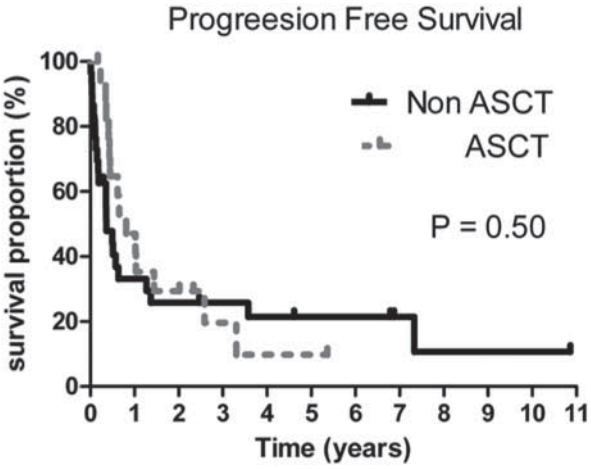
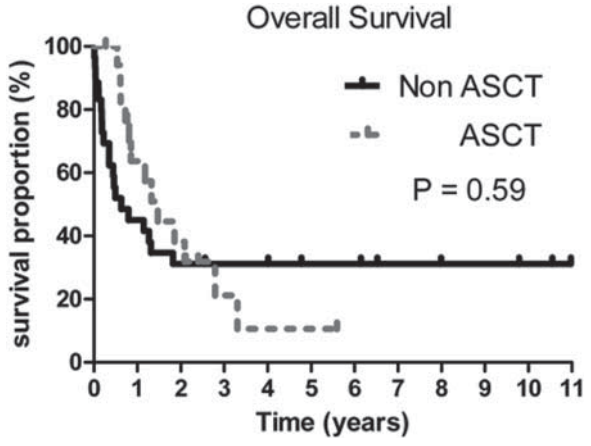
Autologous stem cell transplantation (ASCT) is considered the standard salvage therapy for relapsed or refractory Hodgkin (HD) and non-Hodgkin lymphoma (NHL). 18F-fluoro-deoxyglucose positron emission tomography (FDG-PET) was largely used to explore the predictive value in early evaluation of treatment and at the end of therapy. We want to evaluate the role of FDG-PET performed before autologous stem cell transplantation (ASCT).
Between January 2005 and August 2010 in our centre were performed 122 autologous stem cell transplantation (ASCT) in patients with relapsed or refractory Hodgkin (HD) and non Hodgkin lymphoma (NHL). Eighty one out 122 transplanted patients are retrospectively evaluable to have performed an FDG-PET before ASCT.
The median age was 42 years (range 19-67 years); patients received autografts for NHL (44 pts) and HD (37 pts). At time of transplant 48 pts (59%) were in complete remission (CR), 5 pts (6%) in partial remission (PR) and 28 pts (35%) with refractory disease. The FDG-PET before ASCT was negative in 48 pts

(59%) and positive in 33 pts (41%). After a median period of observation of 27 months (range 0-82 months) the overall survival (OS) was 94% in the FDG-PET-negative group and 40% in the FDG-PET positive group (p: 0.000). The progression free survival (PFS) was 94% and 42% (p: 0.000) respectively for pts with FDG-PET negative and positive after a median time of observation of 22 months (range 0-82 months).
After three months form ASCT 73 out 81 patients performed FDG-PET for restaging, the FDG-PET was negative in 58 pts (79%) and positive in 15 pts (21%). The PFS was 93% in the FDG-PET-negative group and 27% in the FDG-PET positive group (p: 0.000). The OS was 94% and 32% respectively in FDG-PET negative and FDG-PET positive scans (p: 0.000).
Our results confirm that a negative pre-transplant FDG-PET is associated with a better OS and PFS. Half patients with a pre-transplant FDG-PET positivity could be recovered by high dose therapy and transplant. Moreover patients with a positive FDG-PET after transplantation is associated with a poor prognosis and should be considered for alternative treatments.




**P1145**

**The role of high-dose chemotherapy and autologous stem cell transplantation for relapsed T-cell lymphoma**

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Introduction: The role of autologous stem cell transplantation (ASCT) in the treatment of T-cell lymphoma (TCL) either as consolidation after induction chemotherapy or as a salvage treatment is still a rather controversial issue, albeit the approach is advocated by a party of investigators. We aimed to evaluate the role of ASCT as a salvage treatment for relapsed T or NK-cell lymphoma compared with conventional salvage chemotherapy.
Methods: We made a retrospective comparison in a single center cohort of relapsed TCL who underwent ASCT (n=30) or conventional chemotherapy (n=18) between January 1998 and March 2011.
Results: Twenty four patients had peripheral T-cell lymphomas, not otherwise specified (PTCL-NOS), 14 extranodal NK/T cell lymphoma, 5 anaplastic large cell lymphoma and 5 angioimmunoblastic T-cell lymphoma. Patient characteristics including international prognostic index, histology, Ann-Arbor stage and serum lactate dehydrogenase did not significantly differ between the treatment arms except age (median, 45 [range, 26-70] in ASCT arm *vs.* 61 [range, 20-78] in conventional chemotherapy arm, p=0.001). The complete response (CR) rate seemed to be comparable between two treatment (44.4% in ASCT arm and 50.0% in conventional chemotherapy arm, p=0.77). The median progression free survival (PFS) in those undergoing ASCT and salvage chemotherapy were 9.7 months and 4.4 months, respectively (p=0.50). The median overall survival in each group of patients were 17.7 months and 7.5 months (p=0.59). When we made a comparison on only those with chemosensitive relapse, median PFS and OS for ASCT and conventional chemotherapy group also did not significantly differ (median PFS, 9.7 months and 15.2 months, p=0.29; median OS, 17.7 months and 21.8 months, p=0.28).
Conclusion: There seems to be no significant difference in clinical outcomes including CR rate, median PFS and OS between ASCT and conventional chemotherapy in the treatment of relapsed TCL in this cohort. Considering dismal prognosis in these patients as well as toxicity associated with high dose chemotherapy, our data do not support the role of ASCT in the treatment of relapsed TCL.

**P1146**

**Front-line autologous stem cell transplantation as intensive consolidation in patients with peripheral T-cell lymphomas: multicentre trial in Korea**

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Peripheral T cell lymphomas (PTCLs) are an aggressive subtype of non-Hodgkins lymphoma and they have shown the shorter survival compared with B cell lymphoma. High-dose chemotherapy (HDT) followed by autologous stem cell transplantation (HDT/ASCT) for PTCLs has a potential meaning of consolidating remission for PTCLs. However, the effectiveness of ASCT on distinct conditioning regimens, the optimal transplant timing in the frontline or relapsed are still unclear.
We investigated the clinical outcomes of HDT/ASCT as frontline intensification in 46 patients with newly diagnosed PTCLs except ALK(+) anaplastic large cell lymphoma. Patients underwent ASCT with a uniform conditioning regimen (busulfex, cyclophosphamide and etoposide). The median age was 47 years (17-65). The histological subtypes were 47.9% PTCL-

NOS (n=23), 18.8% anaplastic large cell lymphoma (n=9), 4.2% angioimmunoblastic T cell lymphoma (n=2), 25% extranodal NK/T cell, nasal type (n=12), 2.1% hepatosplenic T cell lymphoma (n=1) and 2.1% enteropathy-associated T cell lymphoma. Thirty patients (62.6%) presented with advanced stage disease (III/IV) and 16 (33.3%) had B symptoms. At diagnosis, 21 patients (43.8%) were classified as high-intermediate/high risk by the age-adjusted IPI (aaIPI) and 10 (20.9%) were classified as high-risk (more than 2 factors) by the prognostic index for PTCL (PIT). Thirty-one patients (67%) could undergo HDT/HSCT and disease status at pretransplant consisted of 23 patients (50 %) with CR and 8 patients (17.4%) with PR. 6 out of 8 patients with PR at pretransplantation improved the response to CR after HDT/ASCT. There was no significant difference of the response rate between CHOP alone or CHOP-like chemotherapy and non-anthracycline-based chemotherapeutic regimen. At a median follow-up of 32.9 months, 23 patients (50%) are alive. The 5-year probability of overall and progression-free survival (PFS) was 48.2 ± 8.1 % and 47.4 ± 8.1%, respectively. However, the 5-year OS and PFS rate in transplanted patients was 57.3± 10.2 % and 55.3 ± 11.3 %, respectively.
Conclusion: Frontline HDT/ASCT in patients with PTCL could be performed with a high response rates and a substantial impact on improving outcome for PFS. Our findings also indicate that busulfex, cyclophosphamide and etoposide is a feasible conditioning regimen in ASCT for PTCLs.

**P1147**
**Substituting BCNU by thiotepa in the conditioning regimen of patients with lymphomas undergoing an autologous stem cell transplantation (team protocol). The experience of one centre**
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Objective: To analyze the feasibility as well as the short term side effects of the substitution of BCNU for thiotepa in the conditioning regimen of patients with lymphomas (Hodgkin's lymphoma – HL and non-Hodgkin's lymphoma – NHL) candidates for a stem cell transplantation (SCT).
Patients and Methods: From May 2011 to December 2011, 13 patients were included. The TEAM protocol consisted on the substitution of BCNU at a dose of 300 mg/m$^2$ iv on day -6 by thiotepa at a total dose of 10 mg/kg po. Doses of ara-c, etoposide and melphalan were 400 mg/m$^2$ iv (days -5 to -2), 400 mg/m$^2$ iv (days -5 to -2), melphalan 140 mg/m$^2$ iv (day -1). All the patients received granulocyte colony stimulating factor (G-CSF) at a dose of 5 ug/kg sc from day +7.
There were 7 males and 6 females, with a median age of 55.5 (25–67, range) years at transplantation. Eleven patients received an autologous stem cell transplantation (ASCT) (6 males) because of HL (n=5) or NHL (n=6). Time between diagnosis and ASCT was 18 (8–24) [median (range)] months. Ten patients were autografted in second complete remission (CR) and one patient, in partial remission (PR). Two patients (one male and one female) were treated with an allogeneic stem cell transplantation (allo-SCT). Both of them were diagnosed of NHL and were allografted in PR. Time between diagnosis and allo-SCT were 7 and 14 months, respectively. Graft versus host disease prophylaxis (GVHD) was done with Campath 1H and cyclosporine A and tacrolimus.
Results: Non-relapse mortality was 0%. All patients experienced grade 4 hematological toxicity. Median time to recover >0.5 x10$^9$/L neutrophils was 10 (9–15) days and >20x10$^9$/L platelets, 15 (9–18) days. Length of inpatient stay was 20.5 (18–28) [median (range)] days. The most frequent extra-hematological toxicity was gastrointestinal toxicity: 2 patients developed grade 3-4 mucositis (15%) with no patients developing grade 3-4 nausea, vomiting or diarrhea. With a median follow up of 5 (5–7) months after SCT, all patients are alive and in CR from their disease.
Conclusions: The TEAM combination seems to be a feasible and a safe therapeutic option for patients with lymphoma who are candidates for an SCT. Number of patients is too low and follow up too short to further define the effectiveness of this protocol. More patients as well as longer follow up are needed to further evaluate these results.

**P1148**
**Can R-IPI be helpful in selecting patients with diffuse large B-cell lymphoma for autologus transplantation?**
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There is no unique treatment for all patients with diffuse large B cell lymphoma. Different subgroup of patients with DLBCL needs different treatment. In the pre-rutuximab era International Prognostic Index (IPI) was considered to be the most important prognostic factor for survival and the strongest indicator for identification of high-risk patients, who are unlikely to be cured with standard chemotherapy. Having in mind that IPI is based on 5 clinical characteristics (age, performance status, stage, extranodal involvement, LDH level) and it is constructed in the pre-rituximab is clear that R-IPI should be tested in rituximab era to provide any information of its validity.
We retrospectively analyzed unselected population of 80 patients with confirmed diagnose of diffuse large B cell lymphoma treated at University hematology department in the period of 2005-2010. All patients were uniformly treated with R-CHOP regiment as initial treatment with curative intent. There were 80 patients with mean age 54, 5 years (15-84), male 35 and female 45. Older than 60 years were 29 patients (36, 25%). More than half of the patients (42) were diagnosed in advanced stage of the disease. We analyzed five prognostic factors: age, performance status, stage, extranodal involvement, LDH level and through the multifactorial analyses we selected two groups of patients. One with 0 to 2 factors as patients with low risk. Patients with more than 3 factors are considered as high risk. There is statistically significant difference in overall survival between two groups with five years overall survival 70% for low risk patients and 47% for high risk. High-risk patients may be candidates for autologous transplantation as initial treatment, having in mind that in the rituximab era relapses occur very early in the first year and are difficult to be treated. R-IPI score is significant predictor and should be used for risk stratification of patients with aggressive B-cell lymphoma. However, these findings should be validated prospectively in an independent population of patients.

**P1149**
**The influence of CD34+ cells dose in a graft and the number of requisite aphereses for adequate harvesting on the outcomes of autologous haematopoietic stem cell transplantation for refractory and relapsed lymphoma**
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The aim of the study was to evaluate the impact of CD34+ cells dose given in a graft and the number of aphereses required for harvesting adequate number of CD34+ cells on hematopoietic recovery and survival of patients with non-Hodgkin lymphoma and Hodgkin lymphoma treated with autologous haematopoietic stem cell transplantation (autoHCT). We evaluated a total of 124 patients, median age 34 (19-60) years, with refractory and relapsed lymphoma who underwent haematopoietic stem cell mobilization using chemotherapy and G-CSF and first autoHCT between 2006-2010 in our department. The patients

were treated with the median 2 (range 1-6) chemotherapy lines prior to stem cell mobilization. Harvesting ≥ $3x10^6$ CD34+ cells/kg required 1-3 or > 3 aphereses in 92/124 (74%) and 32/124 (26%) patients, respectively. The impact of increased number of aphereses (>3 *vs* 1-3) on platelet recovery was found (p 0.008), whereas the number of transplanted CD34+ did not influence platelet recovery. The 3-year OS for all patients was 79% estimated with the Kaplan-Meier method. In univariate analysis, prognostic factors associated with OS were the number of prior chemotherapy lines (1-2 *vs* >2: 91% *vs* 59%, p 0.002), the number of infused CD34+ cells (>4 *vs* 3-4x$10^6$/kg: 85% *vs* 59%, p 0.039) and the number of aphereses required for harvesting CD34+ cells (1-3 *vs* >3: 86% *vs* 61%, p 0.016). In multivariate analysis, only the number of prior chemotherapy lines remained statistically significant (HR 6.5, p 0.005) for OS. We conclude that platelets recovery after autoHCT depends on the number of aphereses required to obtain the adequate number of CD34+ cells in patients with lymphoma. We found an association between the number of aphereses, as well as CD34+ cells dose in a graft and survival of lymphoma patients after autoHCT, but only the number of chemotherapy lines prior to autoHCT is the independent prognostic factor for survival.

**P1150**

**Outcome of autologous transplantation for patients with mantle cell lympoma: A single center experience**

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Introduction: Mantle cell lymphoma (MCL) represents about 6% of all non-Hodgkin›s lymphoma (NHL) cases and is associated with a disappointing long term prognosis. Median overall survival (OS) ranges between 3 to 6 years. Although high response rates can be achieved with firstline immuno-chemotherapy, remissions are of relatively short duration. Thus, intensive treatment regimens including high-dose chemotherapy followed by autologous hematopoietic stem cell transplantation (HSCT) have been explored in the past.

Methods: We retrospectively investigated the outcome of patients with MCL who had undergone HSCT at the Heinrich-Heine-University of Duesseldorf. Between 1993 and 2011, 31 patients (6 female, 25 male) received an autologous transplant for MCL. All patients except of one presented with advanced disease (stage III and IV) at diagnosis. The median age at time of transplantation was 56 years (range, 34-75). 18 patients received an autologous transplant in first remission, whereas 13 patients underwent HSCT as second line salvage therapy.

Results: Median follow up of surviving patients is 1023 days (range 175-2845). Sixteen patients (52%) are alive. There was no TRM. Sixteen patients (52%) have relapsed at a median time of 406 days after autografting (range, 54-1673). Of these 13 (81%) have died either of lymphoma (10) or infection (3) after allogeneic transplantation. Two more patients died in CR of secondary myelodysplastic syndrome. The 2 year and 5 year PFS were 68% and 35% and the 2 year and 5 year OS were 83% and 69%, respectively.

Univariate analysis of pre-transplantation variables revealed a significantly inferior outcome for patients relapsing within 6 months following first-line chemotherapy (OS p<0.0001 and PFS p<0.0001). Of note, patients achieving a pre-transplant second remsission after late (>6mo) relapse had the same OS after HSCT as patients in first remission.

Conclusion: Autologous transplantation is an effective first line treatment option for patients with MCL. Patients with early relapse after initial chemotherapy should be treated with innovative treatment approaches including allogeneic transplantation and/or novel agents.

**P1151**

**Busilvex in combination with etoposide and melphalan as conditioning regimen in autologous haematopoietic cell transplantation for lymphomas: efficacy and safety**

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During the last two years, given the carmustine unavailability, in our centre we have used the combination of Busilvex 3.2 mg/kg/day for 3 days, Etoposide 400 mg/m$^2$/day for 2 days and Melphalan 140 mg/m$^2$ (BuEM) since 2009. Sixty-seven patients (pts) have already been enrolled, 40 males, 27 females, aged Deltam 33 (15-65) years, diagnosed with Hodgkin‘s (HL) (41) and non Hodgkin‘s lymphoma (NHL) (26), after Deltam 3 (2-9) lines of treatment. Disease status at AHCT was complete remission (CR) 17, primary refractory (PrimRef) 37 (chemosensitive 14/37) and relapse (Rel) 13 (chemosensitive 5/13). To date 59 pts have been evaluated for at least +3 months' (m) disease response [Deltam follow up 12 (1.4-31.6)m]. Engraftment time was 10 (8-31) for neutrophils and 11 (6-150) days for platelets in 56/59 pts. In terms of engraftment, we have observed 1 graft failure followed by allogeneic double cord blood transplant, 1 late engraftment rescued by a second infusion of autologous cells and 1 haemophagocytic syndrome post 2nd AHCT for refractory relapsed HL. Toxicity was acceptable with bacteraemias grade II (WHO), no serious pulmonary infections and moderate, resolved liver veno-occlussive disease in 1 pt. According to response assessment at +3m in CR were 32/59 (54%), stable disease 9, partial remission (PR) 3 and progression 13 pts which subsequently reached to 21/59(35.6%). At last follow up, 50(84.7%) pts are alive and 32 in CR. No death was attributed to treatment related toxicity. For the whole cohort of pts the estimated progression free survival (PFS) is 65.9% at 2 years and for those in +3m CR 96% (vs 30.9% for not CR, p<0.0001) at 2 years. Chemosensitive disease (PFS: 93.5% *vs* 36.7%, p<0.001) and pre-transplant disease status (PFS: CR 100%, PrimRef 54%, Rel 54.5%, p=0.03) were significant favorable factors. PFS was significantly higher in HL rather in NHL (74.8% *vs* 51.4%, p=0.017) and notably in chemoresistant HL pts (HL: 55.6% *vs* NHL: 9.1%, p=0.0005). The achievement of +3m CR was significant factor for higher PFS in both chemosensitive (100% *vs* 68.6%, p=0.02) and resistant disease (85.7% *vs* 20%, p=0.0017). These preliminary results are encouraging, offering high PFS and a significant rescue probability in chemoresistant pts. Toxicity was acceptable so far, but long term adverse events are not still accessible.

**P1152**

**The results in patient with lymphoma treated with high-dose mitoxantrone and melphalan followed by autologous stem cell transplantation**

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Background: Evidence from randomized trials standard dose chemo ± radiation ± versus high dose chemotherapy (HDT) have been shown that HDT is superior to provide event free survival (EFS) and overall survival (OS) advantage in some of the patients with lymphoma. Several conditioning regimen have been used but most experiences with BEAM and CBV. None is superior to another. We report the results of the HD mitoxantrone (60 mg/m$^2$) and melphalan (180 mg/m$^2$) followed by autologous stem cell transplantation (ASCT). Documented literature on this issue is limited.

Patients and Methods: We conducted in this study 43 patients with relapsed / refractory HL (%40) and NHL (%60) using HDT followed by ASCT. Only patients sensitive to salvage chemotherapy were eligible for the protocol.

Results: Prior to HDT and ASCT, 46% of the patients were in complete remission and 54% were in partial remission. Treatment-related and nonrelated mortality rate at day 100 was 8% for non-Hodgkin's lymphoma and %15 for Hodgkin's lymphoma patients. At a median follow-up of 22 months (range, 1–42), 14 patients in complete remission, 7 patients relapsed/progressed and 7 Hodgkin's lymphoma patients and 15 non-Hodgkin's lymphoma died. The estimated 2-year disease freee survival and overall survival were 38% and 48% in Hodgkin's lymphoma and 38% and 62% in non-Hodgkin's lymphoma, respectively. Factors predicting overall survive were response to conventional salvage therapy and stage prior to salvage therapy. When compared to patients achieving partial remission, patients who attained complete remission prior to HDT/ASCT had a significantly higher probability of 2-year overall survive (81% *vs* 40%).
Conclusion: HDT consisted of mitoxantrone and melphalan followed by ASCT is an effective treatment for some relapsed lymphomas. Although the number of patients was limited, the outcome was encouraging, suggesting a prospective multicentre study is necessary for this protocol.

**P1153**
**Retrospective multicentre analysis of conditioning regimens prior to autologous stem cell transplantation for non-Hodgkin's lymphoma**
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Objectives: Optimal conditioning regimen prior to autologous stem cell transplantation (ASCT) has yet to be determined in patients with non-Hodgkin's lymphoma. We investigated the outcomes of patients underwent ASCT with several conditioning regimens.
Methods: A retrospective analysis of patients with advanced disease in first remission, or relapsed/refractory patients who received ASCT at 5 hospitals in Korea was performed. Total 68 patients were analyzed. The median age was 49 years (range, 15-71). Histology was diffuse large B cell lymphoma (DLBCL) in 31 (46%) patients, peripheral T cell lymphoma in 14 (21%), mantle cell lymphoma in 7 (10%), follicular lymphoma in 3 (4%), and others in 13 (19%) patients. BEAM/BEAC, BuCyEto (busulfan, cyclophosphamide, and etoposide), FluTBI (fludarabine plus total body irradiation), or BuMelEto (busulfan, melphalan, and etoposide) conditioning regimens were used for ASCT in these patients.
Results: Three-year event-free survival (EFS) and overall survival (OS) were 52.3% and 65.3%, respectively. Patients who received BuCyEto and BuMelEto regimens (3yr-OS rates, 73.5% and 75.0%, respectively) had better OS compared to those with BEAM/BEAC regimen (3yr-OS rate, 33.8%) (P=0.038). Younger age, stage I-II disease, high risk in first remission or second remission after first relapse, achievement of complete remission after ASCT, and no occurrence of veno-occlussive disease (VOD) showed better outcomes in terms of EFS and OS after ASCT. Response status before ASCT did not have any impact on survival after ASCT. Bone marrow (BM) involvement did not have an impact on EFS or OS, which means ASCT after achievement of CR with front-line chemotherapy might have survival advantages in patients with BM involvement. Patients with advanced DLBCL who underwent ASCT in first remission (3yr EFS and OS, 66.7% and 77.8%, respectively) had better survival than those underwent ASCT in second or third remission after relapse (3yr EFS and OS, 36.9% and 50.5%, respectively). It suggested that up-front ASCT in first remission might have survival benefit in DLBCL patients with advanced disease. VOD was occurred in 4 patients and all of them received BuCyEto chemotherapy as conditioning regimen (4/46 patients, 8.7%).
Conclusion: BuCyEto and BuMelEto are effective conditioning regimens before ASCT in lymphoma patients. It is prefer to undergo up-front ASCT in DLBCL patients with advanced disease who achieved remission with first-line chemotherapy.

**P1154**
**BEAM and BEAC with or without Rituximab are comparable and effective preparative regimens for patients with B-cell lymphoma undergoing autologous haematopoietic stem cell transplantation**
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High-dose chemotherapy with carmustine, etoposide, cytosine arabinoside, and melphalan (BEAM) or cyclophosphamide (BEAC) followed by autologous hematopoietic stem cell transplantation (ASCT) is considered as a standard therapy for many patients with high-risk/relapsed non-Hodgkin Lymphoma (NHL). Whether addition of rituximab to the preparative regimens (R-BEAM or R-BEAC) improves outcome in patients previously exposed to rituximab, is not clear.
We retrospectively evaluated our center's experience of ASCT between January 2007-December 2009 and attempted to compare efficacy, outcome, and toxicity of BEAM with or without rituximab (± R) (n=34) and BEAC with or without rituximab (± R) (n=19) in patients with high-risk/relapsed NHL. Out of 53 patients (median age 58 y; range 25-72 y), 44 patients received preparative regimen with rituximab (R-BEAM n=29; R-BEAC n=15) and 9 patients without rituximab (BEAM n=5; BEAC n=4).
Overall survival (OS) and disease-free survival (DFS) of the entire cohort (n=53) at 2-years were 88% and 67% respectively.
The probability of OS of BEAM/BEAC group (85%) versus R-BEAM/R-BEAC group (89%) and DFS of BEAM/BEAC group (67%) and R-BEAM/R-BEAC group (71%) were similar at 2 years (p=0.11 and 0.19 respectively).
The probability of OS of BEAM group (± R) (90%) and BEAC group (± R) (89%) and DFS of BEAM group (± R) (63%) and BEAC group (± R) (60%) were also similar at 2 years (p=0.86 and 0.78 respectively).
No statistically significant difference was noted in engraftment kinetics, grade III-IV toxicities, and average lengths of stay among different groups.
We observed significantly increased charge and cost with BEAM (± R) compared to BEAC (± R) (median charge: BEAM $71,000; R-BEAM $75,000; BEAC $44,000; and R-BEAC $63,000) which was attributed to the higher inpatient charges for high-dose melphalan.
In conclusion, addition of rituximab to the preparative regimens did not add any benefit to our patients undergoing ASCT for B-cell NHL. It is possible that universal use of rituximab with prior therapies might have abrogated the benefit of R-BEAM/R-BEAC over BEAM/BEAC. In the era of managed care and increasing cost scrutiny, BEAC may have an advantage especially when the outcomes and safety of the regimens were comparable. However, this hypothesis needs to be tested in a larger cohort with longer follow-up.

**P1155**
**Autologous stem-cell transplantation in patients with relapsed or refractory aggressive B-cell lymphoma: influence of prior exposure to rituximab on outcomes**
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Background: Several studies have shown that the use of highly effective rituximab (R)-containing primary therapy in Diffuse

Large B-cell Lymphoma (DLBCL) makes it more difficult to salvage patients who are refractory or who relapse. To date, autologous stem-cell transplantation (ASCT) is the reference treatment for these patients. In the present study, we have evaluated the impact of prior exposure to R on the ulterior results of ASCT.
Patients and Methods: We have retrospectively analysed 106 patients (pts) with DLBCL (n=92) or grade 3B follicular lymphoma who consecutively received ASCT as salvage therapy at our centre between May 1990 and September 2010. Median age was 51 years (14-70). The analysis was performed according to whether patients had (n=51, "R+" group) or had not (n=55, "R-" group) received R prior to ASCT.
Results: Patients in the R+ group had higher complete remission (CR) (92% *vs* 69%, p=.003) and overall response (OR) (81% *vs* 67%, p=.045) rates than patients in the R- group. In multivariate analysis, factors with significant influence on CR rates were: sex (female), disease status at transplant (CR), number of prior chemotherapy lines (<2), and prior exposure to R (yes). The median follow-up was 42 (2-113) and 97 (2-219) months in the R+ and R- groups, respectively. Patients in the R+ group had a significantly better progression-free survival (PFS) (72% *vs* 52% at 5 years, p=.048) and overall survival (OS) (85% *vs* 61% at 5 years, p=.02) as compared with patients in the R-group. However, the only factors that significantly influenced PFS and/or OS in multivariate analysis were: non-response after first-line treatment (RR: 4.4 [PFS]), refractory disease at transplant (RR: 6.3 [PFS], 13.8 [OS]) and year of transplant > 2000 (RR: 4.6 [OS]. Analyzing separately the R+ group, both PFS and OS were better in patients who received R with the first-line therapy (83% and 93% at 5 years, respectively) than in patients treated with R only in the salvage setting (53% and 70%, respectively), although the differences did not reach statistical significance (p=.09 and .06, respectively).
Conclusions: Our retrospective single-centre analysis indicates that ASCT is still an effective option for patients with chemosensitive relapsed or refractory aggressive B-cell lymphoma pretreated with R-containing first-line or salvage chemotherapy.

**P1156**
**Positioning of the high-dose therapy and autologous stem cell transplant in refractory and/or relapsed Hodgkin's lymphoma – single-centre study**
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Background: Hodgkin lymphoma (HL) is treated with the intent to cure the disease in all stages, and long-term survival exceeds 85 percent for all stages. Historically, patients who relapsed after a full course of chemotherapy had a low chance for cure with second-line treatment, with the duration of initial remission as significant predictor of subsequent response and relapse-free survival.
Aim: The aim of this study was to evaluate clinical course of relapsed and refractory patient with HL who were treated with high dose therapy combined with autologous stem cell transplant (SCT).
Patients and Methods: We investigated 45 (25F/20M) patients - aged 30.9 (20-46) years - with relapsed or refractory HL who were initially treated with ABVD therapy during the period 2005-2010. In the course of relapse or refractory disease, all patients received DHAP as salvage, and BEAM as conditioning regimen due to autologous SCT.
Results: There were 13 patients in II CS defined as early unfavorable group, while 32 patients had advanced disease in III and IV CS. Treatment outcome on the initial therapy was: CR in 19 (42.2%), PR in 3 (6.7%), SD in 11 (24.4%), and PD in 12 (26.7%) of patients. Among clinical characteristics bulky mediastinal mass was present in 60%, lymphadenopathy in 88.8%, splenomegaly in 13.3%, hepatomegaly in 8.9%, and lung infiltration in 35.6% of patients. Laboratory parameters demonstrated anemia in 55.6%, lekocytosis in 42.2%, as well as lymphopenia and trombocytosis in 71.1% patients. Low albumins were present in 71.1%, and elevated ESR, CRP and beta2microglobulin in more than 90% of patients. Median duration of mobilzation (G-CSF 10 µg/kgbm) was 6 days, CD34+ cuont was 8.97 range 2-29 (x10E6/kgbm), median CD34+ cell viability was 78.6%. Median duration of period for WBC engrftment was 13.9 (9-19) days, and for PLT was 13.1 (9-20) days. Survival after auto SCT was 31 (1-45) months, and overall survival (OS) was 86 (12-144) months. Overall response rate at d+100 was 68.8%, with 48% of CR. Also, bulky mediastinal mass, leukocytosis, lymphopenia, trombocytosis, and anemia had negative impact on OS. Using multivariate Cox regression analysis the most predictive prognostic factor for OS was treatment outcome at day +100.
Summary/Conclusion: High-dose therapy and autologous SCT are adequate therapeutic options for poor prognosis refractory/relapsed patients with HL, and posttransplant CR could be the most predictive factor for long term survival.

**P1157**
**MIPI score and day 15 post transplant lymphocyte count predict survival in patients with mantle-cell lymphoma treated with rituximab-hyperCVAD followed by busulphan and melphalan ASCT**
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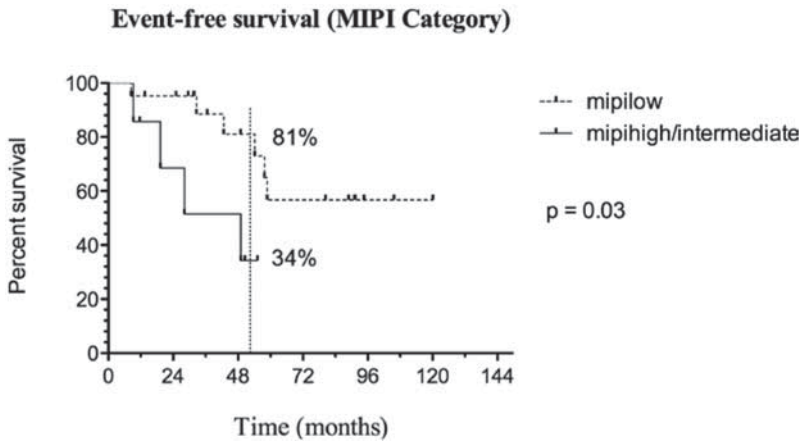
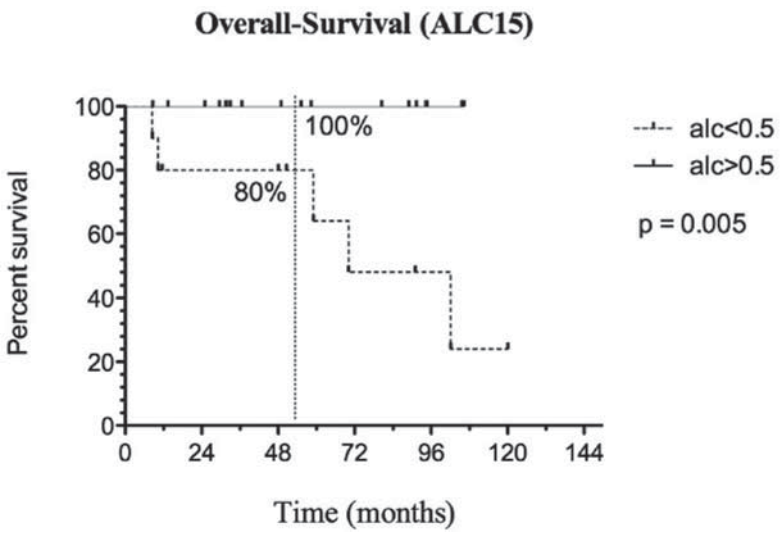
Objectives: To analyse the impact of mantle cell lymphoma (MCL) international prognostic index (MIPI) and absolute lymphocyte count on day +15 (ALC15) post autologous stem cell transplantation (ASCT) on patient outcome after Rituximab+HyperCVAD followed by ASCT with Busulphan (Bu)+Melphalan (Mel) conditioning as initial therapy in patients with MCL.
Background: Improved complete remission (CR) and overall survival (OS) rates have been reported in MCL patients treated with R-HyperCVAD regimen (JCO 2005). ASCT provides a further incremental improvement (Ann Hematol 2006). MIPI predicts outcome in non-ASCT treatment of MCL, but is not associated with outcome in patients treated with R-HyperCVAD (Blood 2008). ALC recovery after ASCT predicts OS in MCL patients treated with CHOP/CHOP-like regimens (BMT 2006), but has not been assessed in those treated with R-HyperCVAD and ASCT.
Method: We retrospectively analysed the outcome of 28 patients with MCL enrolled in our treatment protocol of R-HyperCVAD followed by BuMel ASCT. One patient in CR post R-HyperCVAD refused ASCT. MIPI was calculated pretreatment. ALC15 was obtained from serial blood counts after ASCT.
Results: CR was achieved after R-HyperCVAD in 92.6%. 100% of pts were in CR after ASCT. At a median follow up of 53 months, estimated EFS (Event Free Survival) was 68 % and OS was 92.7%. EFS and OS rates at 53months in patients with ALC15 $>0.5x10^9$/L (n=17) were 94% and 100% compared to 34% and 80% in those with ALC15 $<0.5x10^9$/L (p=.02 and .005 respectively). These rates in patients with low MIPI (n=21) were 81% and 95% compared to 34% and 85% in those with intermediate/high MIPI (p=.03 and .004 respectively). TRM occurred in one patient by day 100. There have been no therapy-related MDS/AML.
Conclusion: R-HyperCVAD followed by ASCT with BuMel conditioning achieves high CR rates and durable remissions in patients with MCL, in particular those with low MIPI and early ALC recovery. Patients with high MIPI or poor ALC recovery justify consideration of post-ASCT maintenance therapy.

[P1157]




**P1158**

**Autologous haematopoietic stem cell transplantation for lymphoma: experience in Shiraz**

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Hematologic malignancy currently represents the main indication for HSCT. Clearly, autologous and allogeneic HSCT are established therapies in many of hematologic malignancies. High dose therapy (HDT) supported by autologous HSCT are the preferred choice for lymphoma and multiple myeloma.
Between Jan 2004 and Nov 2010, 117 patients with diagnosis of lymphoma including Hodgkin's lymphoma (HD) and non-Hodgkin's lymphoma (NHL) underwent autologous peripheral blood stem cell transplantation in our center in Shiraz University of medical sciences. Patients were treated by intensive chemotherapy followed by reinfusion of non-cryopreserved autologous stem cells. The pretransplant conditioning chemotherapy regimen was CEAM (lomustin 200 mg/m$^2$, etoposide 1000 mg/m$^2$, cytarabine 1000 mg/m$^2$ and melphalan 140 mg/m$^2$) for Lymphoma.
During this time, 59 HD patients with median age 27 years (range; 16-50), 58 NHL patients with median age 29 years (range; 18-55) underwent autologous peripheral blood stem cell transplantation. The median time to platelet count >20×10$^9$/L was 14 days (range; 9-31).The median time to absolute neutrophil count (ANC) >0.5×10$^9$/L was 11 days (range; 8-21). All patients have engrafted and there were not graft failure in this study group. 100 days transplant related mortality rate was 3.4% in HD and 5.2% in NHL group respectively.
Our data reflects the important role of HDT followed by HSCT in improvement of outcome for a lymphoma patients of in our center. We concluded high dose therapy with this modified conditioning regimen rescued with non- cryopreserved auto SCT is safe and effective method that is feasible in our lymphoma patients.

**P1159**

**High-dose therapy and autologous stem cell transplant in indolent lymphoma. Review of patients with long-term follow-up at a single centre**

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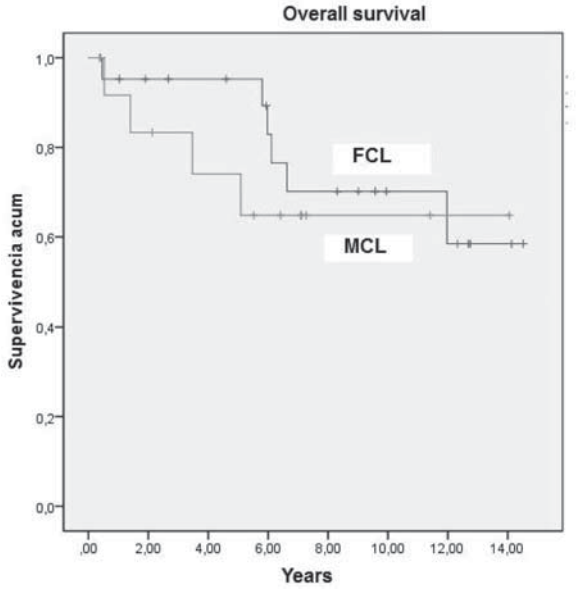
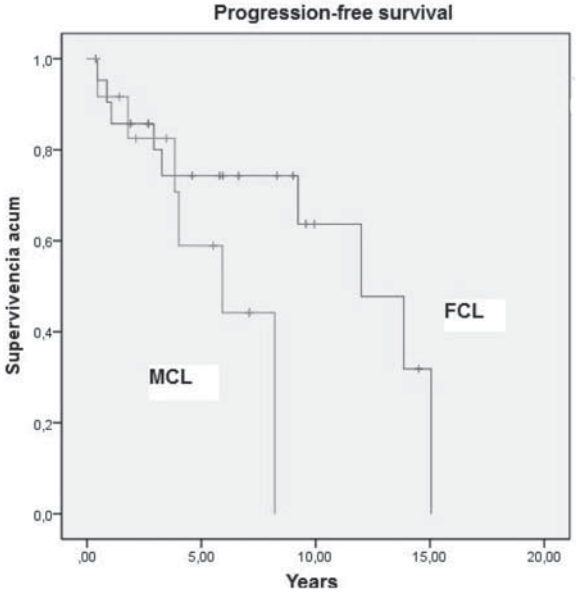
Background: Indolent lymphoma (IL) is a heterogeneous diseases group whose treatment is not really well established. The principal aim of therapy is to prolong overall survival (OS) with the best quality of life. Rituximab, a relatively non-toxic therapy, when combined with chemotherapy lengthens progression-free survival (PFS) and OS, both as initial therapies as at

recurrence. In other side, myeloablative therapy & autologous stem cell transplant (ASCT) has showed to prolong PFS in follicular lymphoma (FL) and mantle-cell lymphoma (MCL), although has not showed to prolong OS for the most of these patients (pt).
Patients and Methods: We analyzed retrospectively 36 consecutive pt transplanted in our centre for a period of 16 years (y), (1995-2011). They had a median age of 54 y [28-68y]. Twenty-one pt (58%) were diagnosed as FL, 13 pt (36%) as MCL and 2 (6%) as other histology. Before ASCT, median of therapies received were 2 [1-6], and 17 pt (47%) were heavily pre-treated (3 or more lines of chemotherapy); 26 pt (72%) were in complete remission (CR) and 10 pt (28%) in partial remission (PR). Thirteen pt (36%) had not received rituximab before ASCT. All of conditioning therapies were non-radiotherapy contained (i.e. BEAC, BEAM, and BUCY).
Results: The best response after ASCT was CR in the 97% pt and PR in 3%. Ninety percent pt in PR achieved a CR after ASCT. After a median follow-up of 6 y, 16 pt (44%) have relapsed, although they have received new rituximab-based therapies. Twenty-five pt were alive at the last follow-up, 22 in CR (61%). Sixteen pt (44%) have never relapsed after ASCT and they are actually in continued CR. Median PFS was 9.2 y. and median OS was not reached. There were no differences considering treatment with rituximab before ASCT (yes or not), status at ASCT (CR or PR), or number of lines of chemotherapy before ASCT (1, 2 or 3 or more). About histological subtype, there were also no differences in OS (FL 11.4 y *vs* MCL 10 y, p=0.5) or PFS (FL 10.3 y *vs* MCL 5.6 y, p=0.11) Seven pt have been diagnosed of a second malignancy, 3 solid tumours (8%) and 4 haematological malignancies (11%) at 4 and 6 y medium time after ASCT.
Conclusions: In our experience, ASCT plays a role in 2000´s in IL. It achieves high CR rate with prolonged PFS & OS. Relapses after ASCT can be rescued with new therapies including rituximab and novel agents in lymphoma, as lenalidomide, bortezomib and new monoclonal antibodies. So, long OS as aim in IL is nearer than ever.

**[P1159]**




**P1160**

**Busulfan, melphalan and etoposide followed by autologous stem cell transplantation on patients with non-Hodgkin's lymphoma: multicentre study from Consortium for Improving Survival of Lymphoma (CISL) in Korea**

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Background: High dose chemotherapy followed by autologous stem cell transplantation (ASCT) has become the standard approach for relapsed or high risk non-Hodgkin's lymphoma (NHL). Several different high dose therapy (HDT) conditioning regimens have been used for NHL, such as BEAM, BEAC and CBV. Carmustine is an active drug in the HDT of NHL but the supply of carmustine is limited in some countries including Korea The purpose of this prospective multicenter phase II study was evaluate the efficacy and safety of iv busulfan/melphalan/etoposide regimen as a conditioning regimen for high dose chemotherapy in the patients with relapsed or high risk NHL.
Methods: Patients with relapsed or primary refractory NHL or chemosensitive high risk NHL underwent high dose chemotherapy followed by ASCT at 12 centers in Korea. The conditioning regimen consisted of iv busulfan 3.2 mg/kg/day i.v. on days -8, -7 and -6, etoposide 400 mg/m$^2$/day i.v. on days -5 and -4 and melphalan 50 mg/m$^2$/day i.v. on days -3 and -2.
Results: Fifty one patients were enrolled onto the study. Main subgroups were DLBCL (n=25, 49%) and T cell lymphoma (n=19, 37%). All patients had successful stem cell engraftment with a median time to neutrophil recovery of more than 500/mm$^3$ of 10 days (range, 2 to 30 days). Platelet recovery of more than 20,000/mm$^3$ was seen after a median of 10 days (range, 2 to 51 days) with delayed recovery in one patient.
Treatment related toxicities included nausea/vomiting in 28 patients (55%), diarrhea in 28 patients (55%) and mucositis in 33

patients (65%), which were grade I or II in the majority of cases. There were no VOD and treatment related death. Forty one patients (80%) achieved a complete response 1 month after ASCT, while three patients showed progressive disease. At a median follow up of 14.7 months, 21(41%) patients exhibited a relapse or progression, while 11 patients had died of disease and one patient had died of heart failure.
The estimated 2-year overall and progression free survival for all patients was 64% and 40%, respectively.
Conclusion: This preliminary analysis suggests that conditioning regimen of i.v. busulfan/melphalan/etoposide would be well tolerated and effective in patients with relapsed or high risk NHL. Accordingly, this regimen may be regarded as an important treatment option to substitute for BEAM regimen.

**P1161**

**Ibritumomab tiuxetan (Zevalin) and BEAM chemotherapy as conditioning regimen for autologous stem cell transplantation in resistant/relapsed non-Hodgkin lymphoma patients: evaluation of toxicity and efficacy**

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Objectives: To evaluate the safety and the efficacy of combined ibritumomab tiuxetan (Zevalin)–BEAM as conditioning regimen for ASCT for resistant/relapsed lymphoma patients, attempting to augment the anti-lymphoma effect without increasing toxicity.
Methods: conditioning regimen consisted of Carmustine (300 mg/m$^2$ day -6), Etoposide (150 mg/m$^2$ days -5 to -2), Cytarabine (400 mg/m$^2$ days -5 to -2) and Melphalan (140 mg/m$^2$ day -1). Zevalin was administered at a dose of 0.4 mCi/kg, 14 days prior to the start of conditioning. The study included 17 patients, median age 51 (38-68) years, transplanted at our centre for non Hodgkin Lymphoma (LDBCL 9; follicular lymphoma 6; small lymphocytic 1; splenic marginal zone 1) transplanted at our centre between May 2008 and October 2011. The patients received a median of 2 (1-3) lines of treatment before ASCT. Disease status at transplant was: 2ª CR, 7 patients (41%); 3ª CR, 1 patient (6%); 1ª PR, 3 patients (18%); 2ª PR, 2 patients (12%); chemosensitive relapse, 4 patients (23%). In one patient it was his second ASCT.
Results: The median of infused CD34+ cell was 3.28 (range 1,92-5.7)×10$^6$/Kg and after patients received G-CSF for a median of 7 (3-13) days. Engraftment was achieved in all patients. The median time for neutrophil engraftment (>500/mm$^3$) was day +11 (8-14) and for platelet engraftment (>20,000/mm$^3$) was day +14 (11-18). Infection during neutropenia developed in 15 patients (bacteremias mostly). 15 patients developed mucositis (grade I: 2, II: 6, III: 5 and IV: 2) and 4 patients received parenteral nutrition. 4 developed liver toxicity and 9 gastrointestinal toxicity (nausea/vomiting/diarrhea). The median days of hospitalization was 24 (21-36) days. 8 patients were admitted to hospital during post-ASCT period because of infectious causes, with a median of 1 (1-6) hospital admissions. At last follow up, 14 patients are alive: 8 in CR, 1 in PR, 3 in relapse and 2 are awaiting reevaluation. Transplant related mortality at day 100 is 0%. 3 patients died as consequence of their disease. With a median follow-up of 15 months (1-59), the estimated 5-year overall survival is 75±12.7% and progression-free survival is 50.6±13.4%.
Conclusions: With the limitation of the small number of patients and short follow-up, the addition of Zevalin in the high-dose chemotherapy regimen given prior to ASCT is feasible and with no added toxicity. This approach may have an advantage in reducing relapse rate and improve outcome.

**P1162**

**Zevalin (Yttrium-90 ibritumomab tiuxetan) and BEAM conditioning chemotherapy (Z-BEAM) is superior to rituximab and BEAM (R-BEAM) prior to autologous stem cell transplantation in patients with mantle-cell lymphoma**

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Introduction: The outcome of patients with mantle cell lymphoma (MCL) remains poor. Maintenance treatment with the anti-CD20 antibody rituximab as well as high-dose chemotherapy (HDCT) followed by autologous stem cell transplantation (ASCT) have improved survival of MCL patients. Based on this, we investigated the role of ibritumomab tiuxetan (Zevalin), a 90Yttrium labeled CD20 targeting antibody, as part of the conditioning regimen in MCL patients undergoing ASCT.
Methods: This retrospective single center study analyzed 27 consecutive MCL patients receiving HDCT with rituximab and BEAM (R-BEAM) or Zevalin prior to BEAM (Z-BEAM) followed by peripheral ASCT. Zevalin was given at 14.8 MBq/kg on day -14 prior to ASCT while Rituximab was administered on the first day of BEAM conditioning.
Results: 18 patients received HDCT with R-BEAM and 9 patients had Z-BEAM. The median age of the patients was 56 years (range 35-67). 85% had advanced-stage disease (stage III or IV), with 74% showing bone marrow infiltration at diagnosis. 16% had blastoid variant and 84% had classical MCL. Before ASCT, the rates of complete remission (CR) in the R-BEAM and Z-BEAM groups were 33% and 28%, and partial remission was seen in 67% and 72%, respectively. The mean number of transplanted CD34+ cells were 3.75 and 4.28 x 106/kg (p=0.319). The median day of engraftment after ASCT was 11 days for leukocytes and 14 days for platelets recovery, with no differences between the two regimens. There were no early treatment related deaths in either group, and no differences in toxicities and infection rates were observed. The median follow-up for MCL patients who received R-BEAM or Z-BEAM was 30.5 months and 25.8 months, respectively. Progression after ASCT occurred in 50% in the R-BEAM and in 22% in the Z-BEAM group (p=0.231). 3-years OS in the R-BEAM versus Z-BEAM groups was 69% and 89% (p=0.532), and 3-years PFS was 27% and 78% (p=0.194), respectively.
Conclusion: To the best of our knowledge, this is the first pilot study comparing two cohorts of MCL patients, one treated with Z-BEAM, and the other one with R-BEAM as conditioning regimen prior to ASCT. High dose chemotherapy with Z-BEAM tended to yield better 3-years OS and PFS compared with R-BEAM, without increased toxicity. Our data indicate that in MCL patients Z-BEAM followed by ASCT could possibly be more effective than R-BEAM, without added toxicity. This approach should be tested in a prospective randomized trial.

**P1163**

**Autologous stem cell transplantation for follicular lymphoma: a nation-wide survey with a very long follow-up**

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Background: Follicular lymphoma (FL) is the second most common lymphoma entity characterized by often widespread presentation, excellent response to treatment but high-risk of recurrence sometimes with a histologic transformation.

Recently monoclonal antibodies notably rituximab have revolutinized the treatment of FL although transplant approaches can be still considered in some clinical scenarios as a treatment option. We have gathered follow-up data from a nation-wide series of FL patients treated with ASCT in 1994-2002 with a minimum follow-up of the living patients of eight years.
Patients: Between 1994 and Dec 2002 altogether 91 patients received ASCT for FL in five Finnish transplant centres. The number of transplanted patients ranged from six to 26 per centre. There were 47 females and 44 males with a median age of 50 years at transplant. Advanced disease stage was observed in 78% of the patients at diagnosis, bone marrow involvement in 46% and B symptoms in 26% of the patients. Disease status at transplant included first complete remission (CR) or partial remission (PR) in 43% of patients and 2nd CR or PR in 50% of the patients. A histological transformation had been observed in 33% of the patients before ASCT and an early relapse (<2 years) in 30% of the patients, respectively. Twenty-five patients (28%) had received rituximab before ASCT.
Results: The median follow-up of all patients was 94 months from ASCT (range 0-168). Altogether 34 patients (37%) have died. Twenty patients (22%) died due to lymphoma and 14 patients (15%) from other reasons. Three patients died due to early complications (<100 d from ASCT), three patients from late infections and five patients due to another malignancy (acute myeloid leukemia 2, myelodysplastic syndrome 1, lung cancer 1, gastric cancer 1). Overall survival (OS) at 10 years was 65% and event-free survival 57%, respectively. OS at 10 years was comparable in patients transplanted after the first-line therapy (70%) compared to patients transplanted later (57%) (p=0.11).
Conclusions: ASCT is an efficient therapy also in relapsed follicular lymphoma with a promising OS. Even with prolonged follow-up the incidence of late complications seems to be acceptable.

**P1164**

**Clinical follow-up of paediatric high-risk patients with recurrent CD20 positive B-cell malignancies treated with the trifunctional antibody FBTA05 (anti-CD3 x anti-CD20)**

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Treatment options for pediatric patients with B cell malignancies refractory to standard therapy are limited and patients display poor prognosis. Thus novel treatment approaches are urgently required. Here we follow up nine children diseased with Non Hodgkin's Lymphoma (NHL) or acute leukemia being treated with FBTA05, a trifunctional anti-CD3 x anti-CD20 antibody in compassionate use. According to patient history, all children presented extensively pretreated and refractory to standard treatment (radiation, chemotherapy) including rituximab and allogeneic stem cell transplantation (five patients) before treatment with FBTA05.
Within individual treatment schedules, two different treatment strategies were studied. Either weekly applications of FBTA05 up to 300 µg to dissect GvL and GvHD and induce cellular immunity after allogeneic transplantation or daily applications with escalated doses up to maximal 1,000 µg to reduce tumour burden or to eradicate minimal residual disease.
In context of allogeneic transplantation FBTA05 was followed by DLI in two patients. Here, in one case of NHL FBTA05 and DLI were additionally combined with lenalidomide within a second and third treatment cycle. All other children were treated stand alone with FBTA05.
Eight of the nine children displayed a clinical response: two stable diseases, one partial remission and five complete responses (CR). Of note, in one of these patients a molecular CR was achieved after stand alone treatment with FBTA05.
In follow up overall survival (OS) is currently in the range of 59 up to 829 days. Three out of these eight responders died. Two children due to relapse or tumor progression (OS 59 days to 129 days), one due to suspected pulmonary embolism (OS 829 days). The other patients still sustain in complete or partial remission.
Within follow up analysis human anti-mouse antibodies (HAMAs) were detectable only in one case appearing four weeks after start of FBTA05 therapy.
Interestingly, two additional applications of FBTA05 could be administered safely. Thereby HAMAs disappeared four months later. Graft-versus-host disease (grade III-IV) could be observed in two patients (in one case after DLI), and resolved by further immunosuppressive therapies.
Taken together, despite poor clinical prognosis of pediatric patients refractory to standard therapy follow up of 9 patients treated with FBTA05 revealed a favorable clinical outcome including partial and complete remissions.

**P1165**

**Histone modifications and DNA demethylation enhance the cytotoxicity of combined nucleoside analog-DNA alkylating agents in human lymphoma cell lines**

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Purpose: Hemapoietic stem cell transplantation (HSCT) is used for treatment of lymphoma patients. To optimize an efficacious and safe pre-HSCT conditioning therapy, we investigated the possible synergistic cytotoxicity of busulfan (Bu), melphalan (Mel) and gemcitabine (Gem) in lymphoma cells. We also determined if epigenetic changes would enhance [Bu+Mel+Gem] cytotoxicity.
Methods: Cells were exposed to drugs individually or in combinations and analyzed for proliferation by the MTT assay, cell cycle by flow cytometry, and protein levels/modifications by Western blotting.
Results: We used ~IC10 drug concentrations (57 µM Bu, 1 µM Mel, 0.02 µM Gem) which individually did not affect the proliferation of J45.01 cells. Their combination resulted in 50% inhibition of cell proliferation relative to untreated controls. Reduction to about half concentrations (20 µM Bu, 0.7 µM Mel, 0.01 µM Gem) did not inhibit proliferation, but when the histone deacetylase inhibitor suberoylanilide hydroxamic acid (0.6 µM SAHA) was added it resulted in a marked (~65%) inhibition. Similar inhibitory effects of [Bu+Mel+Gem+SAHA] were obtained in B-cell lymphoma, CML and AML cell lines. The cytotoxicity of [Bu+Mel+Gem+SAHA] correlated with activation of the ATM-CHK2 pathway, phosphorylaton of KAP1, and epigenetic changes such as methylation and acetylation of histone 3 and protein poly(ADP-ribosyl)ation. Drug-induced activation of apoptosis was shown by increase in Annexin V-positive cells, proportion of cells with sub-G1 DNA content, and cleavage of PARP1 and caspase 3. The relevance of epigenetic changes was further shown by the induction of DNA methyltransferases DNMT3A and DNMT3B and global DNA methylation after [Bu+Mel+Gem+SAHA]. The observed increase in DNMT3A and 3B was reversed by the addition of 5-aza-2'-deoxycytidine (DAC) with a consequential further enhanced cell kill.
Conclusions:

1) Combining the nucleoside analog Gem with Bu+Mel results in synergistic cytotoxicity in lymphoma cells.
2) Addition of SAHA sensitizes lymphoma cells to these drugs and inclusion of DAC to the [Bu+Mel+Gem+SAHA] regimen further increases drug efficacy in cells that exhibit increased DNMT3A and 3B.
3) SAHA and DAC are chemical sensitizers for Bu+Mel+Gem via induction of epigenetic changes which open up chromatin structure for more efficient DNA alkylation.
4) This study justifies clinical trials using [Bu+Mel+Gem+SAHA±DAC] as pre-HSCT conditioning in lymphoma.