Graft versus host disease – clinical

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Proteomic patterns are of superior prognostic value to EBMT score

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Objectives: Allogeneic stem cell transplantation (SCT) is an established treatment for many severe disorders of hematopoiesis. However, allo-SCT is associated with substantial morbidity and mortality. In 1998, Gratwohl et al. defined a risk score for CML patients (pts) depending on 5 pretransplant variables. Recently, this score was validated also for other disease entities. One focus of proteomics is the identification of biomarkers, preferably predictive for disease prior to clinical manifestation. We have previously applied proteomics to identify a urinary polypeptide patterns (PP) predictive for developing acute GvHD (aGvHD). The aim of this study was to investigate whether the PPs are able to predict overall outcome after allo-SCT and compare these findings to those of the EBMT score.

Methods: This is a retrospective study from Hannover Medical School. The dataset includes all SCT performed between 2003 to 2008 for which relevant PP data are available. PP data from urine samples collected in the time frame of aGvHD from day +7 ongoing are correlated with both the EBMT score as well as clinical parameters (overall survival (OS), aGvHD, non-relapse mortality (NRM), relapse rate and mortality).

Results: 172 patients have been identified for whom PP data are available. According to their GvHD match on PP, patients are grouped to high, low or intermediate risk group. 64 patients (37%) belong to the low risk group (LR) for aGvHD compared to 75 patients (44%) who are classified as high risk (HR). The median EBMT risk scores for both the HR as well as the LR group are identical with 4 points. OS compares favorably for the LR group with an OS of 73% vs 52% for the HR group (p=0.01). Fifty-one vs 22 patients developed aGvHD in HR and LR group (p<0.01), respectively. aGvHD ≥ II was observed in 27 vs 9 patients of these groups (36% vs 14%, p<0.01). NRM is higher in the HR group (31% vs 11%; p<0.01) but relapse rates and relapse mortality are similar for both groups (p=0.61 and p=0.71, respectively).

Conclusion: Risk stratification according to GvHD-match based PP is not only able to predict aGvHD but also reveals different prognostic groups for OS and NRM. In comparison to the EBMT Score, pp-based prediction shows higher accuracy. Since proteomics is a new method which has been available only at a few centers in recent years, further multicenter analyses are essential to determinate the value of pp-based prediction of complications and outcome in HSCT.

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The presence of activating killer immunoglobulin-like receptors on donor natural killer cells is critical to the successful outcome of unrelated donor allogeneic haematopoietic stem cell transplantation for thalassaemia

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Host-derived antigen-presenting cells (APCs) can be lysed by natural killer (NK) cells and are considered to trigger the pathological cascade leading to graft-versus-host disease (GvHD). With the aim of elucidating the effect that the complete absence of activating killer immunoglobulin-like receptors (KIRs) on donor NK cells could have on hematopoietic stem cell transplantation (HSCT) outcome, we investigated whether the NK cells of donors possessing only the deletion variants of activating KIRs (2DS4*003-*009) were less capable of eliminating patient APCs than donor NK cells expressing one or more activating KIRs.

A study was performed on a homogeneous cohort of 93 transplanted thalassemia patients to evaluate whether the incidence of acute GVHD (aGVHD) was higher in patients transplanted from HLA-identical unrelated donors completely lacking functional activating KIR genes. Moreover, we evaluated the ability of donor NK cells to eliminate patient APCs in vitro by comparing the cytotoxic effect of NK cells from donors with and without functional activating KIR genes.

The activating KIR, KIR2DS4, was present in 80% of the donor and recipient pairs. The 2DS4*001 full-length allele was present in 19.9% and the non-functional deletion variants for the activating receptor KIR2DS4 were present in 80.1%. Patients transplanted from donors only carrying non-functional deletion variants of KIR2DS4 had an even higher risk of developing aGvHD than patients transplanted from donors carrying at least one activating KIR gene. Six of the 10 patients who developed severe aGvHD had received grafts from donors carrying only non-functional deletion variants of 2DS4 [hazard risk (HR) 6.2, 95% confidence interval (CI) 1.5-24.9, P=0.01]. All six patients died of complications associated with aGvHD. A possible explanation is that donors who only carry deletion variants of 2DS4 do not express activating KIRs on the NK cell surface. This lack of expression probably lowers the ability of NK lymphocytes to exert their cytotoxic effects on tumour cells and hematopoietic-derived targets, including host APCs. In this study of patients transplanted for β-thalassemia major, the lack of activating KIRs on donor NK cells significantly reduced NK cell cytotoxicity against APCs of the respective recipients (apoptosis 36% vs 17.5%, P<0.001), thereby increasing the risk of GvHD (HR 4.5, 95% CI 1.6-12.3, P=0.005) and transplantation-related mortality (HR 4.0, 95% CI 1.5-14.2, P=0.03).

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Prophylaxis and treatment of graft-versus-host disease after allogeneic stem cell transplantation: a survey of centre strategies by the European Group for Blood and Marrow Transplantation

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Clear recommendations on indications for allogeneic haematopoietic stem cell transplantation (HSCT) have been established. In contrast, the techniques used have remained poorly standardized. Reported outcomes vary markedly, and it would be important to know, whether this is due to differences in treatment procedures. EBMT performed a survey among its member centres about their strategies in preventing and treating graft versus host disease (GvHD). Eighty-one centres from 23 countries participated. The main prophylactic regimens used for HSCT with myeloablative conditioning were cyclosporine (CsA) + methotrexate (MTX) in 86%, CsA + mycophenolate mofetil (MMF) in 6%, tacrolimus + MTX in 6%, ex vivo T-cell depletion (TCD) in 2%, and other in 7% of the centres. The initial CsA dose/kg/day was most commonly 3 mg (50%), range 1-12.5 mg. The target blood concentrations during the first week after transplantation ranged between 80 and 500, and at 2 months between 60 and 400 mikrog/l. The typical duration of CsA prophylaxis was most commonly 180 days (44%), range 56-365 days. Four doses of MTX were given in 70% and three doses in 30% of the centres. Leucovorin rescue was given in 43%. ATG was included for some subgroups in 79%, alemtuzumab in 25%, corticosteroid in 10%, and TCD in 24% of the centres. The prophylactic regimens in reduced intensity transplantations were CsA + MMF (69%), CsA + MTX (38%), CsA (22%), in vivo TCD (18%), tacro + MMF (10%), ex vivo TCD (3%), and other 2%. All centres used corticosteroid as first line treatment of acute GvHD. The treatment was started at first signs likely to be caused by GvHD (17%) or for grade II or higher (83%). The decision to treat was based on clinical signs only in 70% of the centres while in 30% histological documentation was needed. The initial daily dose/kg was most commonly 2 mg (71%), range 1-20 mg. The dose per kg/day indicating corticosteroid resistance was most commonly 2 mg (72%), range 1-15 mg. The most widely used second line treatments were MMF (33%), anti-TNF antibodies (31%), other monoclonal antibodies (16%), ATG (27%), photopheresis (14%), mesenchymal stem cells (7%), alemtuzumab (7%), pentostatin (5%), and “keep going with corticosteroids” (12%).

In conclusion, the present results show marked differences between centres in the prophylaxis and management of GvHD. These findings underline the need for standardization and prospective controlled studies in GvHD prevention and treatment.

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Personalized modelling-based gene selection for developing an early acute GvHD diagnosis support system

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Aim: Because to date there is no a definitive diagnostic blood test for acute graft-versus-host disease (aGVHD) in allogeneic stem cell transplant (HSCT), our objective was to validate a gene expression array combined with computational intelligence algorithms to identify diagnostic biomarkers of aGVHD at onset of clinical symptoms. We performed a personalized modelling technique to create a model for each objective sample, which was able to discover the most important features specifically for this sample.

Method: This study used a Personalized Modeling based Gene Selection method (PMGS) proposed for macroarray data analysis, figure 1. Fifty-nine HSCT patients were enrolled between March 2007 and July 2009 in transplant unit. We used 26 peripheral blood samples (PBS) from aGvHD (YES) patients that were taken at the time of diagnosis and we selected 33 PBS from patients that didn’t experienced aGvHD (NO). All together YES/NO patient groups comprised a validation set. A macroarray was carried out with TaqMan® Low Density Array Fluidic. We selected 47 candidate genes involved in immune network and inflammation pathogenesis.

Results: In the personalized model the selected frequency of some gene transcripts was significantly high, which means they can be recognized highly representative of the data pattern. For example CASP1, FOXP3, ICOS, CD52 are the most important genes for sample 20. A leave-one-out cross validation procedure was performed to investigate the robustness of the PMGS method over the training set: in 29 runs the personalized best subset was selected 29 times (100%). From a biological point of view, the results are reliable. It is noteworthy that in our study a set of genes, indicated by computational analysis, included same mediators of Th2 response All these were strongly down-regulated in aGVHD (YES) setting, suggesting absence of control mediated by Th2 cells. FOXP3 surrogate marker of T regulator cells was the best informative indicator of alloreactive syndrome. The ROC curve for the major informative genes (Foxp-3 and ICOS) were showed in figure 2.

Conclusions: This study demonstrated that the proposed integrated methodology for the personalized selection of gene diagnostic targets and their use for diagnosis of aGVHD results in a satisfactory 92% accuracy over independent test data set of HSCT population. The author are working to develop a user-friendly software package for clinical testing from medical staff.

Fig. 1 diagram of personalized modeling based gene selection method (PMGS)

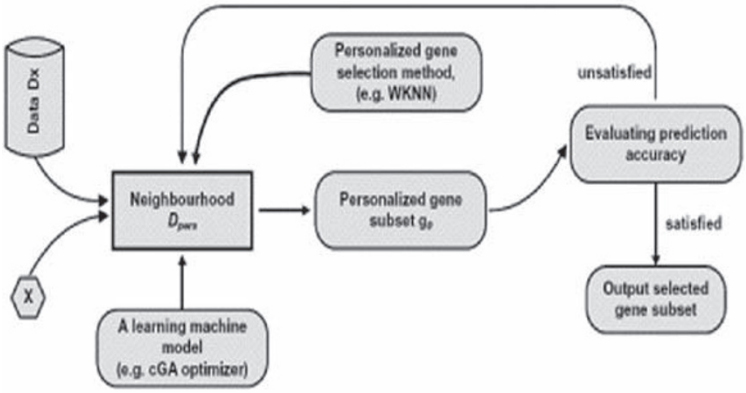
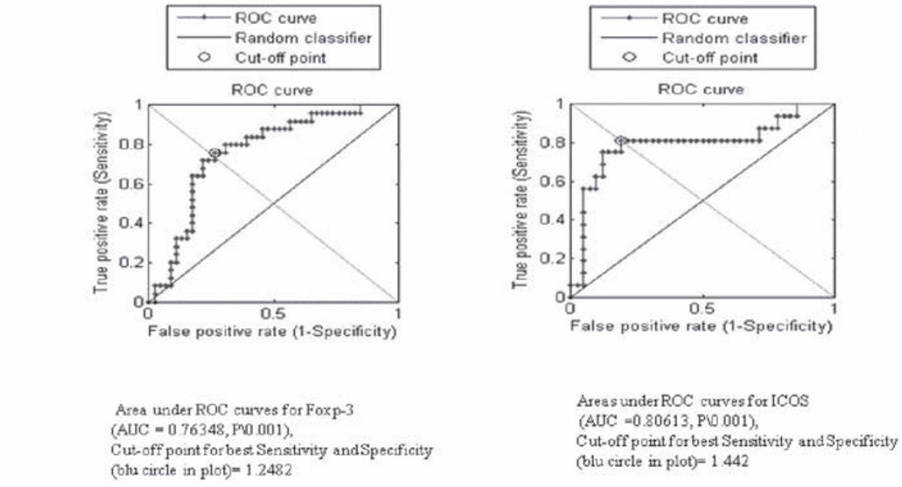


Fig. 2



Area under ROC curves for Foxp-3
(AUC = 0.76348, P0.001),
Cut-off point for best Sensitivity and Specificity
(blu circle in plot)= 1.2482

Areas under ROC curves for ICOS
(AUC =0.80613, P0.001),
Cut-off point for best Sensitivity and Specificity
(blu circle in plot)= 1.442

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Mesenchymal stromal cells for treating steroid-refractory GvHD: scientific basis to improve clinical protocols

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Although the usage of mesenchymal stromal cells (MSC) as therapy for Graft versus Host Disease (GvHD) is constantly increasing, studies on MSC efficacy have been scarcely corroborated by biological analysis of patient response to cell infusion. We report the immunological monitoring of 9 patients with steroid-refractory GvHD, receiving multiple doses of MSCs. GvHD presented as acute in 5 cases and chronic in 4 cases. After MSC therapy, 2 patients showed complete response (CR), 4 patients showed partial response (PR) whereas 3 patients did not respond (NR) to MSC infusion.

To better comprehend the immunomodulatory effects of MSC infusions, we studied GvHD plasmatic markers, inflammatory cytokines and CD4+ T-cell subsets circulating in the peripheral blood (PB) of enrolled patients before MSC infusion and at day 7, 14 and 28 after cell therapy.

In accordance with clinical observations, in CR patients we observed a dramatic decrease of three validated GvHD plasmatic markers TNFRI, IL2Ra and elafin (Paczesny S et al. Blood 2009) to the mean levels of Healthy Donors (HD). In particular, at day 28 after therapy, TNFRI decreased of 2 times, IL2Ra levels decreased of 1.9 times and elafin decreased of 2.3 times.

Moreover, investigating the effect of MSC infusion on lymphocyte counts, we observed in both CR patients a significant decrease in CD3+ and CD4+ lymphocyte counts in the PB. Interestingly, after MSC infusions, CD4+ T-cell subsets changed significantly: Tregs increased and Th1 and Th17 populations decreased. In particular, Th1/Treg ratio decreased of 3.5 times and Th17/Treg ratio decreased of 3.6 times. Correspondingly, patient symptoms also gradually improved, suggesting an association between GvHD clinical course and CD4+ T-cell imbalance.

In accordance with the decrease of Th1 CD4+ T cells in the PB of CR patients, we observed a valuable decrease of IFNg plasma concentrations, which reached the levels typical of HD. Contrary to CR patients, in PR patients we observed a transient decrease of GVHD plasmatic markers and Th1/Treg, Th17/Treg ratios, while NR patients showed stable or even increasing levels of all analysed plasmatic and cellular markers.

In summary, despite its limited size, the present study suggests that MSCs, upon infusion, are able to convert an inflammatory environment to a more physiological one, both at a cellular level, promoting the expansion of circulating Tregs, and at a molecular level, diminishing inflammatory cytokines.

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TCR-like positive neutrophil reconstitution following haematopoietic stem cell transplantation

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In allogeneic hematopoietic stem cell transplantation (HSCT) the complete or near-complete ablation of host immunity and subsequent establishment of donor-derived immunity, is a prerequisite for successful engraftment and long-term outcomes.
Specific immunity relies on recombinatorial immune receptors, which are traditionally thought to be restricted to lymphoid cells. Our recent identification of variable TCR-like α-β immune receptors (TCRLn) in neutrophils indicates the existence of a variable host defense machinery beyond lymphocytes. This notion has recently been confirmed by the demonstration of TCRβ locus rearrangements in thymic neutrophils from transgenic mice and evidence for TCRγ-delta expression by human eosinophil granulocytes.
Here, we aimed at exploring the pathophysiological potential of the TCRLn+ subpopulation during early reconstitution of the innate immune system following allogeneic HSCT. We investigated whether and to which extent the engraftment of this novel variable host defense system bears the risk of alloreactivity, e.g. graft versus host disease (GvHD). We recruited 20 patients who underwent allogeneic stem cell transplantation. For 16 patients a complete follow-up over 8 months was obtained. Four patients did not survive the early post-transplantation course.
At each time point peripheral blood samples were collected, and CD15+ granulocytes and PBMC were purified and RNA and DNA were isolated. CDR3 spectratyping for analysis of TCRL clonalities (neutrophils) and TCR clonalities (PBMC) was performed. Our results show (i) that the the novel recombinatorial TCRL is fully reconstituted after allogeneic HSCT and (ii) the engraftment period of neutrophils is characterized by dynamic TCRL repertoire changes. In the long-term follow up patients display repertoire diversities similar to those of normal age-matched controls. Around day 180 post transplantation in 16 out of 19 (84%) patients a defined portion of the 25 variable TCRLn β chains was consistently absent. Intriguingly, 12 of 19 (63%) patients developed clinical signs of GvHD.
Taken together, we demonstrate fort he first time, that phagocytes bearing variable immunoreceptors (CD15+/TCRLn+ neutrophils) are succesfully transplanted into a host. This finding potentially provides an as yet unrecognized platform for the identification of novel diagnostic or therapeutic targets and may have a impact on GvHD risk definition.

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Cyclosporine and methotrexate compared with cyclosporine and mycophenolate as GvHD prevention regimens in allogeneic stem cell transplantation from unrelated donors; relative outcomes are dependent on disease status at transplantation

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Allogeneic stem cell transplantation (SCT) from matched unrelated donors (MUD) is a potentially curative approach in patients (pts) with hematologic malignancies and no sibling donor. Several GVHD prevention regimens have been used but there is no data to support one over the others. We historically used CSA/MTX (n=187 pts). Over the last 3 years we used CSA/MMF (n=92). Median pt age was 52 years (18-73). Diagnoses included AML/MDS (n=152), ALL (n=30), CML (n=12), myeloma (n=24), lymphoma (n=40), others (n=21). The donor was 10/10 (n=191), 9/10 (n=54) or ≤ 8/10 match (n=34). Conditioning was myeloablative (n=58) or reduced intensity/toxicity (RIC, n=221). Non-relapse mortality (NRM) is dependant on regimen toxicity and GVHD. CSA/MMF was less toxic than CSA/MTX. It allowed faster engraftment, day +11 and +14, respectively (p<0.001). Day30 mortality was 2.2% and 10.7%, respectively (p=0.04). Multivariate analysis (MVA) identified high comorbidity index (HR 2.5, p=0.05), advanced disease (HR 6.2, p=0.005), mismatched donor (HR 2.4, p=0.03) and lymphoid malignancies (HR 2.8, p=0.03) as adverse factors for regimen-related mortality. CSA/MMF was protective (HR 0.4, p=0.02). However, CSA/MMF was less effective in preventing grade III-IV acute GVHD; cumulative incidence 29% and 18%, respectively (p=0.005). MVA identified mismatched donor (HR 3.4, p=0.001) and CSA/MMF (HR 2.4, p=0.004) as adverse factors, while RIC was protective (HR 0.4, p=0.01). The net effect was that NRM was equivalent with both regimens. GVHD regimen had major impact on overall survival (OS) when pts were stratified based on disease status. In early stage disease, OS was 65% and 42%, after CSA/MTX and CSA/MMF, respectively (HR 1.9, p=0.05), predominantly due to excess GVHD deaths in the MMF group. In advanced disease, OS was better with CSA/MMF; 20% and 12%, respectively (HR 0.5, p=0.04), predominantly due to less relapses. In conclusion, GVHD prevention regimen has major impact on outcome after MUD SCT. CSA/MMF is a less toxic regimen and allows prompt engraftment, but is less effective in preventing GVHD. In early stage disease, outcome is dominated by NRM and more effective GVHD prevention regimen such as CSA/MTX is needed. In advanced disease both toxicity and relapse increase. A less intensive regimen, that also better preserves GVL, such as CSA/MMF, may be associated with better outcome. The GVHD prevention regimen selected may need to be tailored to pt and disease characteristics.

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Undifferentiated naive and differentiated effector memory T-lymphocytes in chronic graft-versus-host disease: results of a prospective study

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Background: Immune reconstitution has emerged as a general concern in allogeneic hematopoietic stem cell transplantation (HCT). Despite an association of chronic graft-versus-host disease (cGVHD) with alloreactive helper and cytotoxic T-cells, no data on the role of effector T-cells in early phase cGVHD is currently available.
Patients and methods: Between 2007 and 2010, we prospectively characterized cellular immune reconstitution in 101 consecutive adult patients (54 males, 47 females) with a mean age of 42 years undergoing myeloablative (MA, n=62) or reduced-intensity (RIC, n=39) conditioning HCT with related (n=21) or unrelated (n=80) marrow (n=6) or peripheral blood (n=95) stem cell grafts. In patients alive without original disease on day +100 the following lymphoid subsets were quantified by flow cytometry on fresh peripheral blood every 3 months for 2 years: CD4+, CD4+CD45RA+ (naïve T-cells), CD4+CD62L-, CD4+CD45RA+CD31+ recent thymic emigrants (RTE), CD8+, CD8+CD45RA+CCR7-CD27+CD28+ undifferentiated naïve effector memory (EMRA), CD8+CD45RA-CCR7-CD27-CD28- effector memory (EM) T-cells, lin-DR+CD11c+CD123- myeloid dendritic cells (DC1), lin-DR+CD11c-CD123+ plasmacytoid dendritic cells (DC2). Cellular results were correlated with clinical presence or absence of cGVHD at each assessment point.
Results: Seventy (69%) patients experienced cGVHD a mean of 155 (range, 60 to 604) days after HCT. On day +100 CD4+ T-cells, naïve T-cells and RTE were a mean of 266, 81 and 17 cells/ul in all patients. Prior acute GVHD affected only number of RTE (26 vs 8 cells/ul, p=0.01) on day +100. In all patients CD4+ T-cells, naïve T-cells and RTE increased to 427, 122 and 23 cells/ul on day +365, and 516, 177 and 35 cells/ul on day +730 and thus, remained lower compared to healthy individuals. CD8+ T-cells increased from 629 cells/ul on day +100 to 799 cells/ul on day +365 in all patients and thus, remained

elevated compared to healthy individuals.Results in cGVHD compared to patients without cGVHD are shown in the table. No significant differences in myeloid and plasmacytoid dendritic cells were observed between these cohorts.
Conclusions: CD4+ T-cell deficiency is pronounced in the first years after allogeneic HCT. In patients with cGVHD undifferentiated naïve effector memory T-cells remain significantly lower up to 2 years after HCT whereas effector memory T-cells are elevated indicating rapid reactivation and stronger cytolytic activity after antigen re-encounter.

Subset	Day +100 cGVHD vs no	Day +180 cGVHD vs no	Day +730 cGVHD vs no
CD4+ cells/ul	314 vs 168, p=0.003	394 vs 219, p=0.03	515 vs 519, NS
Naive CD4+ cells/ul	102 vs 38, p<0.001	121 vs 46, NS	188 vs 131, NS
RTE cells/ul	25 vs 6, p<0.001	30 vs 6, p=0.05	36 vs 31, NS
CD8+ cells/ul	777 vs 329, p=0.03	724 vs 411, NS	720 vs 549, NS
EMRA %	13 vs 32, p<0.001	11 vs 19, p=0.06	11 vs 29, p=0.02
EM %	17 vs 8, p<0.001	21 vs 11, NS	20 vs 14, NS
Ratio EMRA/EM abs	2.9 vs 87.8, p=0.002	7.3 vs 5.5, p=0.07	6.8 vs 13.9, p=0.08

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10 mg alemtuzumab may efficiently prevent severe GvHD in sibling PBSCT for acute leukaemia and higher doses are not required

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The optimal dose of in vivo administrated alemtuzumab in the allogeneic transplantation setting has not been defined. Recently, a multicenter, prospective British trial suggested that 30 mg alemtuzumab should be the standard (Chakraverty et al., Blood 2010;116:3080). Although this might be true in patients transplanted with active CD52+ lymphoproliferative disease, in which alemtuzumab clearance is faster, analysis of the subgroup of patients with acute leukemia suggested that a further reduction is possible. We report our experience on 37 patients with high risk diseases, mainly acute leukemias (AML 23, ALL 10, MDS RAEB 1, CML blast crisis 1, AA/PNH 2), who underwent sibling (49%) or unrelated (51%) PBSCT (35 pts) and received a total dose of only 10 to 20 mg Campath-1H as part of the conditioning and post-transplant cyclosporine without methotrexate. The neutrophil and especially the platelet engraftment was rapid. There were only 2 grade III-IV acute GvHD cases which occurred in unrelated transplants of the Campath-10 cohort. Chronic GvHD developed in 6 cases (17%) and was limited to skin in 5 of them. The single case of severe liver GvHD beyond d+100 was in a HbsAg+ patient/donor pair who received 10 mg alemtuzumab. After a median follow up of 371 days (59-1191), 70% patients are alive and in CR (Karnofksy 100%) and 11 died (TRM n=6, relapse n=5). From the 5 pts relapsed, 3/5 were at advanced stage at transplant and 4/5 underwent sibling HCT with the higher (20 mg) alemtuzumab dose. With the 10 mg alemtuzumab schedule (5 mg/d at d-2 and d-1) we achieve at day of transplantation low but still lymphotoxic alemtuzumab serum concentrations (176 ng/ml), while levels decline fast thereafter and at engraftment nearly no Campath antibody remains in the patient's serum (ELISA performed at BioAnaLab Ltd, Oxford, UK). Taken together, the results of Chakraverty et al., Bertz et al. (BBMT 2009;15:1563) and our current results clearly indicate that in patients with acute leukemia, 10 mg alemtuzumab combined with only CsA post-transplant may efficiently prevent severe acute and chronic GVHD after sibling PBSCT and higher doses are not required. The optimal minimal dose of alemtuzumab for preventing GvHD in the unrelated setting probably ranges between 10 mg to 20 mg.

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CD3/T regs ratio in donor graft predicts aGvHD and immunological recovery after allogeneic peripheral stem cell transplantation

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The therapeutic efficacy of allogeneic stem cell transplantation (alloSCT) for hematological malignancies relies on the graft versus leukemia (GvL) effect exerted by the donor CD3 cells, but an uncontrolled 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. In our study we analyzed the CD3/Tregs ratio in donor grafts and determined its impact on the aGvHD and immunological recovery (evaluated on recovery of CMV-specific CD8+ T lymphocytes). We analyzed 48 myeloablative alloSCT; patients were transplanted with unmanipulated peripheral blood stem cells from an HLA identical related donor (n=33) or an HLA identical unrelated donor (n=15). Median age was 32 years (r:18-58); diagnoses were acute myeloid leukaemia (n=41), acute lymphoblastic leukaemia (n=7). We used fluorochrome-conjugated tetrameric complexes of HLA to monitor recovery of CMV-specific CD8+ according to the patient's HLA.The median CD3 and Tregs dose administered was 250 (r:125-550) and $10x10^6$/Kg (r:1-51), respectively; the median CD3/Tregs ratio was 34 (r:4-170). Patients were subdivided into a high CD3/Tregs ratio (>20) group (n=25) and a low CD3/Tregs ratio (<20) group (n=23).
Results: The incidence of aGvHD (grade II-IV) in the low CD3/T regs ratio group was lower than in the high CD3/Tregs ratio group (2/23 vs 12/25, p=.0002). Median CMV-specific CD8+ T lymphocytes were significantly higher in the low CD3/Tregs ratio group than the high CD3/Tregs ratio in the graft at 1 (2cells/mmc vs 0, p<.001), 2 (6cells/mmc vs1, p<.001), 3 (15 cells/mmc vs 3, p<.001) months, respectively. During the three months after transplantation, CMV infection/disease was observed in 2/20 patients in the low CD/Tregs ratio group and in 14/25 patients in the high CD3/Tregs ratio group (p=.001).
Conclusions: We suggest that there is a good correlation between the CD3/Tregs ratio and the incidence of aGvHD and outcome of immunological recovery. In the high CD3/Tregs group, aGvHD and the relative therapy result in poor immune recovery. Instead, in the low CD3/T regs group the high numbers of Treg cells mediate protective effects against aGvHD and the maintenance of an optimal microenviroment for the reconstitution of functional immunity.

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The influence of Rh incompatibility on acute graft-versus-host disease and overall survival in cord blood transplantations in children

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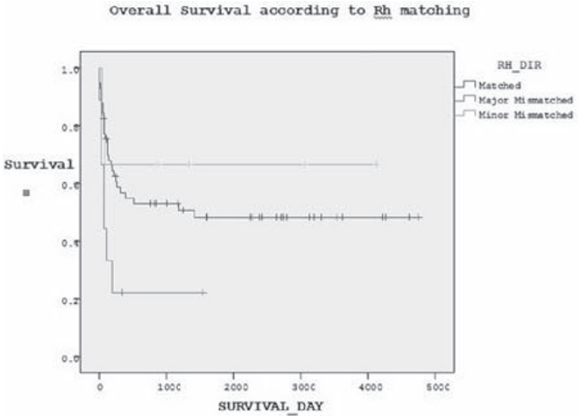
Introduction: The impact of blood type mismatch (MM) on survival following Cord Blood Transplantation (CBT) has been evaluated in several studies with conflicting conclusions. Some studies suggested that ABO MM may have an impact on CBT outcome, but there is no evidence regarding the significance of Rh MM. Our aim was to evaluate the outcome of single CBT in several centers in Israel.
Patients: We analyzed the results of single unit CBT performed in seventy seven children. There were 50 male and 27 female.

Median age at transplantation was 4.2 years (0.1–14.5 years). Forty five patients had malignant hematological diseases and 32 had non-malignant diseases. Fifty eight children received CB from unrelated donors while 19 were transplanted from matched siblings. The median post-thaw total nucleated cell dose was $5.9x10^7$/kg (range 0.5–$68x10^7$/kg). Thirty patients-cord blood pairs were ABO matched. There were major, minor and bidirectional ABO incompatibilities in 26, 18 and 3 patient: donor pairs, respectively. In 56, 9, and 6 patient:donor pairs there were matching of Rh, major MM, and minor MM, respectively (no data in 6).
Results: Neutrophil engraftment at day 30 was 54.1%±6.2%. Platelet engraftment at day 60 was 47.4%±7.2%. Overall survival (OS) at 5 years was 46.3%±6.1%. There was a trend towards better OS in patients with non-malignant disease versus malignant disease (p=0.051). No significant impact on transplantation outcome of several parameters was identified (patient's age and gender, CMV status, ABO MM, type of disease, conditioning regimens, HLA disparity, and cell dose). The outcome of transplantations in which there was major MM of Rh was significantly poorer. Neither hemolytic complications nor delayed erythroid engraftment in the presence of myeloid and platelet reconstitution were reported in any of the groups. Risk factors for engraftment and survival were balanced between patient:donor pairs with matched and MM blood types. Survival at 5 years was 22%, 66% and 50% (p=0.019) in the major MM, minor MM and matched Rh, respectively. Acute graft versus host disease of grade III-IV occurred in 75%, 0% and 25% of patients at risk (p=0.024) in the major MM, minor MM and matched Rh, respectively.
Conclusion: This is the first report of the impact of Rh MM on CBT outcome. The significance of Rh MM in CBT outcome should be evaluated in larger series, and specifically, in the expanding field of double unit transplantations.



P452
Platelet-lysate-expanded mesenchymal stromal cells for the treatment of steroid resistant GvHD
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Mesenchymal stromal cells (MSC) are multipotent cells with immunomodulatory properties, capable to escape immune rejection allowing their use in an HLA-mismatched setting.
We describe a multi-centre experience which started in May 2008 and relied on the use GMP-grade unrelated HLA-disparate donors' bone marrow-derived MSCs, expanded in Platelet-Lysate -containing medium. 11 pts (4 to 15 years) transplanted for malignant (N=8) or non-malignant (N=3) diseases, received iv MSCs for acute or chronic grade I-IV GvHD, which was resistant to multiple lines of immunosuppression. Twenty-one MSC infusions were given to 11 pts. The median dose per infusion was 1.2 x 10^6/kg. Response to treatment was evaluated 28 days after infusion. Overall response (OR) was 75%, with complete response (CR) in 25% of the cases.
We further developed a second part of the study from August 2009 in order to allow an earlier use of MSCs. 15 pts (7 adults, 8 pediatric) aged 1-58 years received iv MSCs infusions for steroid resistant acute or chronic GvHD grade II-IV. All patients developed GvHD after HSCT performed for malignant (N=12) or non malignant diseases (N=3). GvHD presented as acute in 13 cases and chronic in 2, it involved a single organ in 9 pts (6 skin, 3 gut) and multiple organs in the other cases. Pts received 2 to 6 MSC infusions aiming at $1x10^6$/kg recipient body weight MSCs for each infusion. In this series OR was 70 %, and CR 30%. None of the patients affected by chronic GvHD benefited from MSC infusion. Both skin and gut GvHD presented a good response rate, skin showing an earlier response (2 to 4 days) compared to gut (5 to 7 days). Patients with multiple organ involvement seem to show a worse response to treatment. In all 36 treated pts no side effects were observed at MSC infusion. All responder pts could eventually taper ongoing immunosuppression. Seven pts presented GvHD recurrence 2-5 months after infusion. Four pts developed chronic GvHD. 30 out of 36 treated patients are alive and in complete remission from their haematological disease with an average follow-up of 15.5 months from MSC infusion (range 1-29 months). 1 pt is alive with overt relapse of ALL, 2 pts died from GvHD complications, 3 pts died from infectious diseases. The present study underlines the safety of PL-expanded MSC use in children and adults. MSC efficacy seems to be greater in acute than in chronic GvHD, even after failure of multiple lines of immunosuppression.

P453
The impact of donor-recipient matching for non-classical HLA-E and HLA-G, and HSP70-hom (HSPA1L) alleles on HSCT outcome
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Objectives: The present study aimed to determine whether typing for non-classical HLA (HLA-E and HLA-G locus) and non-HLA (HSP70-hom/HSPA1L) alleles may optimize donor-recipient matching be of prognostic value for the outcome of allogeneic haematopoietic stem cell transplantation (HSCT).
Methods: One-hundred patients (F/M=45/55; diagnosed mainly with haematological malignancies (n=87), 10 with inborn errors and 3 with anemia; receiving myeloablative (n=41) or reduced intensity conditioning (n=59) regimen; grafted from sibling (n=45) or unrelated (n=55) donors; transplanted with PBPC (n=87) or BM (n=13)) and their donors were investigated. Patients and their donors were typed for the presence of two HLA-E alleles (*0101, *0103) coding expressed HLA-E molecules, the HLA-G ins/del polymorphism, and the HSP70-hom (HSPA1L) (+2763 G/A) alleles.
Results: Acute graft-versus-host disease (GvHD) (grade III-IV) was more frequent in patients carrying the HSP70-hom (+2763) AA genotype (39% vs 18%, p=0.062, for grade III-IV GvHD). The incidence of aGvHD (grade II-IV) was higher after HLA-E mismatched transplants (67% vs 34%, p=0.040) being matched at the high resolution level for 5 classical HLA loci (HLA-A, B, C, DRB1 and DQB1).
HLA match for 5 classical loci associated with a higher incidence of HHV-6 infection before +100 day after transplantation (>100 copies/10^5 blood cells, 19.5% vs 0%, p=0.069). HHV-6 infection

was not observed after HLA-E mismatched transplants (0% vs 24%, p=0.035).
Fatal complications were more frequent among acute GvHD cases (58% vs 28%, p=0.003). Recipient HLA-E*0103 homozygosity was associated with improved likelihood of patient 3 yrs overall survival (100% vs 55%, p=0.095). This tendency was also seen in patients transplanted with donors carrying the HLA-G del/del genotype (68% vs 48%). The presence of HLA-E*0103, 0103 in the patients and/or HLA-G del/del in the donors significantly facilitated the survival (73% vs 44%, p=0.033).
Conclusion: Extended MHC genotyping (including non-classical HLA-E and G alleles and non-HLA genes) in addition to donor-recipient matching for classical HLA loci is of prognostic value for the outcome of allogeneic HSCT.
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P454

Development of an algorithm to predict acute GvHD and chronic GvHD in HLA-matched sibling, non-T cell depleted haematopoietic stem cell transplants

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Objectives: The EBMT risk score has been developed to predict survival in haematopoietic stem cell transplant cohorts (Gratwohl et al, 2009). Here we develop a scoring algorithm which could be used to predict the occurrence of GVHD (acute and chronic) utilising clinical and genetic variables.
Methods: The cohort consisted of 442 HLA-matched sibling patient and donor pairs collected from Barcelona, Cologne, Newcastle, Paris, Prague, Regensburg, Rostock and Vienna. The transplants were non-T cell depleted. Adult patients were studied where 51.4% had acute leukaemia , 18.6% had chronic myeloid leukaemia (CML) and 14.8% had lymphoma. The pre-transplant clinical variables which acted as predictors of outcome were female donor to male patient, stem cell source and age of patient or age of donor.
Results: After summation of potential predictors, the proportion of cases with acute GVHD II-IV increased as the score increased (Cochran-Armitage test, p=0.009). This trend was much less pronounced for acute GVHD III-IV. After introduction of the score variable into a logistic regression model for acute GVHD II-IV, the odds ratio was 1.28 (95% confidence interval 1.05-1.57, sensitivity 45.9%, specificity 65.3%). For chronic GVHD, additionally acute GVHD and reduced intensity conditioning (RIC) acted as predictors. After summation of the potential predictors, the proportion of patients with chronic GVHD increased as the score increased (Cochran Armitage test, p<0.0005). The odds ratio of the risk score was 1.62 (95% confidence interval 1.33-1.98, sensitivity 70.1%, specificity 51.3%. We are in the process of further developing the models to establish if the addition of selected single nucleotide polymorphisms (SNPs) previously associated with acute GVHD II-IV, e.g. cytokine SNPs IL-13, IL-6, IFN-γ and IL-10, improve predictive power.
Conclusion: Preliminary analysis has suggested that it is feasible to construct a scoring instrument comprising clinical pre-transplant variables to predict occurrence of acute GVHD II-IV. Pre-transplant clinical variables and, additionally, RIC and occurrence of acute GVHD can also be used to predict chronic GVHD. Further work is in progress to establish if predictive power can be improved by addition of selected SNP variables.

P455

Infliximab salvage of allo-HCT patients with steroid refractory acute graft-versus-host disease. A single-centre long-term follow-up study

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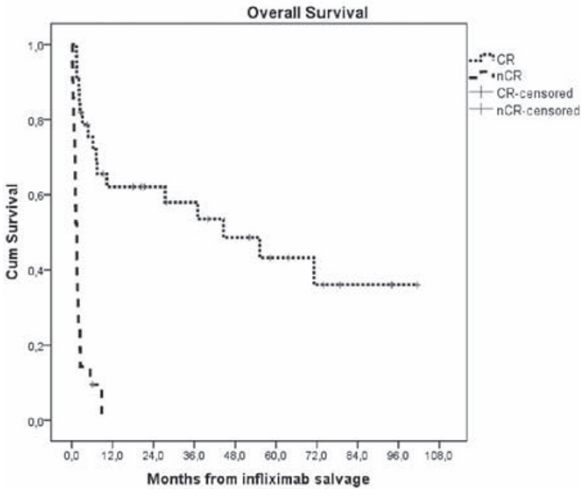
Recipients of allogeneic hematopoietic cell transplantation (HCT) with steroid refractory acute graft versus host disease (aGVHD) have a dismal prognosis. Salvage treatment with the tumor necrosis factor α (TNF-α) chimeric antibody infliximab has been used, but sparse data on outcome exists.
Fifty-four HCT recipients were treated with infliximab for steroid-refractory acute Graft Versus Host Disease (aGVHD) between January 2000 and May 2010. We retrospectively evaluated response and long-term survival after salvage with infliximab.
Median patient age was 50,9 year (25-73), 35 males and 19 females. Conditioning was myeloablative in 16 patients, non-myeloablative HCT in 38 patients. Acute GVHD was considered steroid-refractory if there was no change in overall aGVHD grading within the first week of treatment, or if aGVHD stage in one organ increased one stage during steroid treatment. At first dose of infliximab, 50 patients received prednisolone 2 mg/kg, 4 patients received 1 mg/kg. Infliximab was administered intravenously at a dose of 10 mg/kg once a week. Infliximab was discontinued when GI-GVHD was stage 0, or skin GVHD was stage 2 or less. In case of severe infection, infliximab was postponed or stopped. A median of 3 infliximab doses (range 1-11) was given.
The grading of aGVHD and organ involvement at the time of salvage is shown in table 1.
Median time from HCT or DLI to diagnosis of aGVHD was 117,5 days (range 17-417), and median time from high-dose steroid to salvage with infliximab was 6 days (range 1-70). An overall response (complete remission (CR) or partial remission (PR) without progressive disease (PD) in any organ) was achieved in 42 (78%) of the patients. CR was obtained in 33 (61%) of the 54 patients. The median time from start of infliximab to CR was 21 days (7-121). Overall survival was 31,5% with a

Overall aGVHD grade	N	%
Grade I	0	0
Grade II	17	32
Grade III	25	46
Grade IV	12	22

			Grade (N)			
Organ involvement of aGVHD	N	%	I	II	III	IV
GI only	25	46	0	9	16	0
GI + liver	11	21	0	1	5	5
GI + skin	4	7	0	1	2	1
GI + liver + skin	2	3	0	0	1	1
Skin only	10	19	0	5	1	4
Skin + liver	1	2	0	1	0	0
Liver only	1	2	0	0	0	1

Table 1.



mean observation time of 40,0 months (2,6-101,5). The median overall survival in patients who achieved CR (n=33) was 44,6 months (95% CI = 8,7 to 80,4 months), versus 1,5 months (95% CI = 0,8 to 2,2 months) in patients who did not (n=21) (Fig 2). Causes of death in the non-CR group of patients (n=20) were: GVHD (12), infection (4), relapse (1), and other (3). Causes of death in the CR group (n=17) were: infection (10), relapse (4), and other (3).
Conclusion: Complete remission with infliximab was obtained in 61% of this cohort, and 48% of these were long-time survivors. Infection remains a major problem.

P456
Risk and prognostic factors for acute graft-versus-host disease defined by NIH consensus criteria
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Objectives: The NIH has published consensus criteria (NCC) for the diagnosis of GVHD, which emphasized the manifestations of GVHD instead of the time of onset after SCT. There have been few trials to evaluate both risk and prognostic factors of acute GVHD (aGVHD) defined by NCC. In this context, the present study was designed to investigate risk and prognostic factors for aGVHD according to the new definition of the NCC.
Methods: Between January 2002 to December 2008, 797 patients underwent allogeneic SCT from sibling and unrelated donors for hematologic malignancies. To secure a homogeneous cohort, patients who had undergone previous allogeneic SCT and/or those who received donor lymphocyte infusion were excluded. Ultimately, 767 patients were selected for the analyses of risk factors for aGVHD (the first cohort). Of these patients, 330 developed aGVHD by NCC (the second cohort) and were analyzed to assess the prognostic factors for aGVHD.
Results: The median age was 36 years (range, 15-68). Patients had various hematologic malignancies and were transplanted from sibling (n=485) and unrelated donors (n=282). Conditioning regimens consisted of myeloablative (n=532) and reduced-intensity regimens (n=235). GVHD prophylaxis was based on administering calcineurin inhibitor with short-course methotrexate. The cumulative incidences of aGVHD was 54.9% and aGVHD (n=330) consisted of classic acute GVHD (n=304) and late-onset (n=26) subtypes, and 77 patients who developed chronic features of GVHD before 100 days after SCT were not included as aGVHD. Multivariate analyses revealed significant risk factors for aGVHD were younger age, unrelated donors, and myeloablative conditioning. Multivariate analyses of the second cohort including covariates associated with characteristics of aGVHD at onset revealed GVHD-specific survival was significantly lower in older age and advanced pre-SCT disease status, while skin-only aGVHD and lesser grade of aGVHD at onset were associated with significantly higher GvHD-specific survival.
Conclusions: Our data demonstrate several risk factors for aGVHD defined by NCC and also reveal prognostic factors on the GVHD-specific survival. To our knowledge, this study represents the first one demonstrating the risk factors and prognostic factors for aGVHD by NCC in a large cohort.

P457
Early treatment with ''high dose steroids" for aAllo-immune lung syndromes is associated with improved survival despite the presence of high viral loads of ''common cold viruses"
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Background: Alloimmune lung syndromes (allo-LS), including Idiopathic Pneumonia Syndrome and Bronchiolitis Obliterans, are severe life-treatening complications after HSCT. We recently found that a "common cold" respiratory viral (RV) infection early after SCT is an important predictor for the development of allo-LS and that prolonged administration of immunosuppression, in patients with a respiratory viral (RV) infection, because of aGVHD, paradoxically had a protective effect on the development of allo-LS (BBMT 2010). We therefore hypothesised that despite presence of a RV, alloLD should be treated with 'high dose steroids (HDS)". We prospectively studied the outcomes of the treatment of alloLS with a standard HDS-protocol" and compared this with our historical cohort.
Methods: All patients transplanted between Jan-2004 and Jul-2010, for any indication, were included. All patients were tested for the presence of a RV using qPCR prior to HSCT and subsequently weekly till discharge and after discharge only when having symptoms. AlloLS (IPS, BOS or BOOP: bronchiolitis obliterans organizing pneumonia) was diagnosed according international criteria, excluding infection (except presence of a RV). In 2006 the HDS treatment protocol was introduced when allo-LS was suspected: MP-pulse (10 mg/kg for 3 days), followed by 2 mg/kg/d prednisone, tapered 25% per week till 0.5 mg/kg. After 4 weeks the MP-pulse was repeated, followed by 0.5 mg/kg/d for at least 1 mth. The MP-pulse was repeated, in case of suspicion on residual alloLS, with a maximum of 6 times. Ciclosporine tough levels were maintained between 150-250 ug/L. Before 2006 we were reluctant giving steroids because of the presence of a RV.
Results: 182 pts were included of whom 38 (21%; 15% IPS and 6% BO) developed an allo-LS. Follow up: 36 (1–76 mths). The overall survival was 63% (73% without alloLS, 43% with alloLS). 35/38 of the patients with an alloLS had a proven RV. Cause of death in the alloLS group was 90% TRM and 10% relapse. 10 pts were treated according to old treatment guidelines, while 28 were included in the HDS protocol. The probability of OS was 20% for the "old treatment group" while 54% of the patients within the HDS group survived (p=0.041). The viral load of the RV, expressed as CT-values, remained stable (median value 20: range 17–24) during the treatment with HDS.
In summary: Early initiation of HDS in alloLS improved survival, despite the presence of a RV (infection).

P458
Pharmacokinetics-based optimal dose prediction of mycophenolate mofetil in unrelated haematopoietic cell transplantation: proposal of strategies depending on donor source
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Mycophenolate mofetil (MMF) has been widely used for GvHD prophylaxis after allogeneic stem cell transplantation (allo-SCT). No clear advantage over methotrexate (MTX) has been reported except the fewer incidences of mucositis. Wide inter-individual variation of plasma mycophenolic acid (MPA) levels could mask the benefits; hence, a retrospective evaluation was done on the potential efficacy of individualization depending on MPA-area under the curve for hours 0 to 24 (AUC24).

Methods: Plasma total MPA levels on days 2, 9, and 16 were quantified by reverse-phase HPLC. Median AUC24 at day 16 of 41 patients given 3gMMF/day was 30.4 microg h/ml. Thus, the clinical outcomes in 36 unrelated bone marrow and cord blood transplantations (BMT&CBT) using MMF were analyzed based on AUC24 higher or lower than 30microg h/ml.
Results: Grades II to IV acute GvHD occurred in 29.4% of 34 evaluable patients, which was significantly lower in the high AUC group than the low one (15.8% vs 46.7%, p=0.04). This difference mainly originated from BMT patients (high vs. low AUC; 0% vs. 60%). Extensive chronic GvHD occurred in 13.3% of 30 evaluable patients. Chronic GvHD was significantly suppressed in high AUC group than in low AUC (0% vs. 30.8%, p=0.015). In CBT, no extensive chronic GvHD occurred regardless of AUC level. In BMT, there was a trend toward better OS and DFS at 3y in high AUC group. Whereas patients with low AUC in CBT displayed continuous high OS and DFS with no relapse, those with high AUC showed remarkably poor OS and DFS due mainly to high relapse rate (60%). There was no difference in the incidence of severe diarrhoea or cytomegalovirus-antigenemia depending on AUC. Strong correlation between AUC24 and C2h, MPA concentration at 2hrs after administration, was observed (r^2=0.657, p<0.0001).
Conclusions: This study revealed a new strategy to fully benefit from MMF depending on donor source. The incidence of Grades II to IV acute GvHD in 3944 HLA-matched unrelated BMTs from Japan Marrow Donor Program, wherein MTX was used in the vast majority, is 40%. MMF is particularly useful to surpass MTX in unrelated BMT if AUC24 is higher than 30microg h/ml. In CBT, AUC24 less than 30microg h/ml is good enough for GVHD prophylaxis and is critical to avoid relapse. The benefit would be predictable by a single point monitoring, C2h during the early days after transplantation. Prospective randomized study on individualized administration is necessary to unveil the usefulness of MMF.

P459

Increased production of the inflammatory cytokine IL12p70 by activated human blood dendritic cells is associated with increased severity of acute graft-versus-host disease post-allogeneic stem cell transplantation

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Introduction: The activation status of peripheral blood CD11c+ myeloid dendritic cells (DC) as assessed by CMRF-44 antigen expression is highly associated with the severity of acute GvHD (Transplantation 2007; 83: 839–846). However, very little is known about the function of post transplant DC. We examined the relationship between DC functional properties and the severity of acute GvHD.
Method: Peripheral blood CD11c+ myeloid DC from 12 patients were studied weekly up to 8 weeks post transplant for production of IL2, IL4, INFg, IL10 and IL12 using an intracellular cytokine flow assay. Mixed lymphocyte reactions were used to assess allogeneic immune responses in 5 patients.
Results:IL12 was the only cytokine detected in post transplant DC. Five of 12 patients developed moderate to severe aGvHD (grade II-IV), the remaining 7 patients developed either no aGvHD or only grade I. In comparison with pre-transplant levels of expression, patients with grade 11-1V had up to 4 fold higher percentage of CD11c+ DC expressing IL12 (median 15.1%, range 11.2-20.9%) as compared to patients with grade 0-1 aGvHD in whom there was no change from baseline values (median 6.6% range 2.8-8.9%) p=0.0025. Increased expression of IL12 was observed in CD11c+ DC commencing at day 25 post transplant. Interestingly, analysis of paired samples from 5 patients comparing sorted DC from donors with sorted DC from peripheral blood of transplant patients at day +30 post-transplant showed a marked reduction in the capacity of post-transplant donor DCs to stimulate 3rd party lymphocyte proliferation. None of these patients developed clinically significant aGvHD.
Conclusion: production of IL-12p70 by CD11c+ myeloid DC correlates with severity of GvHD despite apparent defects in the capacity of these cells to elicit 3rd party proliferative responses.

P460

Expression of the chemokine receptor CCR5 on blood CD11c+ CD16+ dendritic cells post-allogeneic haemopoietic cell transplant is predictive for the development of acute graft-versus-host disease

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Introduction: Dendritic cells (DC) are centrally involved in the development of acute graft-versus-host disease (GvHD) following allogeneic hematopoietic cell transplantation (alloHCT). We previously showed that the activation status, as assessed by CMRF-44 antigen expression, of blood CD11c+ myeloid DC is highly associated with the severity of acute GvHD, and that activated DC may be detected in the circulation prior to clinical presentation of GvHD (Transplantation 2007; 83: 839–846). We also reported that there was also a positive correlation between aGVHD and the expression of the chemokine receptor CCR5 on myeloid DC (Blood, 114, Suppl.: 2251, 2009). Because of the phenotypic and functional heterogeneity of the CD11c+ DC population, we further investigated the precise nature of the CD11c+ DC subset expressing CCR5 in the peripheral blood in 24 patients post alloHCT, and correlated the findings with GVHD outcomes.
Methods: Peripheral blood was collected twice weekly up to day 100 post transplant from 24 alloHCT patients. The expression of CCR5 receptor on CD11c+ and CD11c- DC subsets was evaluated using multiparameter flow cytometry.
Results: Eleven of 24 patients developed acute GvHD (4 grade I, 7 grades II-IV), the remaining 13 patients had no GvHD. The percentage of CD11c++ CD16+ DC expressing CCR5 correlated with the development of acute GvHD grades II-IV. The maximum CCR5 expression detected on CD11c+ CD16+ DC in patients developing grade II-IV GvHD (mean 36.0±6.9%, n=7) was higher than in those with grade 0-I GvHD (17.2 ± 3.2%, n=17) (p=0.0153), and occurred prior to the clinical onset of GVHD in 6 of 8 patients with CCR5 levels >20%. Levels of expression of CCR5 on other DC subsets, including CD16-CD11C+ DC, were not preditive for GVHD.
Conclusion: Expression of CCR5 on circulating CD11c+ CD16+ myeloid DC post allo-HCT correlates with the development of moderate to severe GvHD. This observation may indicate altered homing patterns of these cells during the alloimuune response. Detection of raised numbers of CCR5+ CD11c+ CD16+ DC could allow earlier therapeutic intervention.

P461

Higher leucocyte dose in ECP therapy is associated with superior treatment outcomes for chronic GvHD

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Introduction: Extracorporeal Photopheresis (ECP), which involves the induction of apoptosis in harvested peripheral blood leucocytes and subsequent reinfusion, has proved effective in the treatment of steroid-refractory chronic Graft–versus-host-disease (cGVHD). ECP cell treatment dose is variable with no recognised target cell dose. Retrospective review of treatment harvests was performed to assess correlation of leucocyte cell dose and clinical outcome.
Methods: 79 patients with steroid-refractory cGVHD were ECP treated for a minimum 3 months (3m). 63 patients continued ECP for at least 6 months (6m). Treatments were performed using the Therakos XTS™ ECP system (n=1580) or Cellex™ device (n=17). Patients received 2-weekly dual treatments for

3 m then monthly dual treatments for a further minimum 3 m. 73 patients starting ECP were receiving steroids and 61 had cutaneous GVHD. Outcome was measured by change in steroid dose or change in overall skin score after 3 m and 6m of ECP.
Results: Higher mean 3 month total leucocyte dose was associated with an ability to taper steroids within the first 3 months of ECP (n=51) compared to those without steroid reduction (n=16) (795 vs 565 x10^6 cells/kg; P=0.046). Patients achieving at least a 50% steroid reduction at 6 m (n=52) had a higher mean 3m cumulative leucocyte dose compared to non-responders (n=11) (797 vs 519 x10^6 cells/kg; P=0.043) and higher mean cell doses per treatment (61.5 vs 41.3 x10^6 cells/kg; P=0.06,NS). Patients who achieved steroid sparing by 6m (n=18) had the highest 3m cumulative cell doses; (919x10^6 cells/kg; P=0.017). Patients in who steroid taper was possible by 3m and then maintained or further reduced steroid dose (n=45) had higher mean cell doses in the first 3 m compared to steroid taper patients who experienced a reversal of dose reduction (n=7); (64.5 vs 37.2 x10^6 cells/kg; P=0.043). Patients with durable reduction in skin score within 3 m (n=31) had a higher 3m mean cumulative leucocyte dose than patients whose skin score worsened after initial improvement (n=9) (848 vs 561 x10^6 cells/kg; P=0.03).
Discussion: This data provides evidence that the number of harvested leucocytes obtained during ECP is associated with an ability to reduce or cease steroid administration in cGVHD. Higher leucocyte dose was also associated with sustainable cutaneous GVHD improvement. These findings support tailoring treatment based on individual leucocyte harvests to improve likelihood of treatment response.

P462
Improved control of advanced disease and reduced bacterial and fungal infections in patients with everolimus and mycophenolate as GvHD prophylaxis
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Tolerance induction together with sustained disease control are critical for longterm survivors after allogeneic hematopoietic cell transplantation (HCT). Although calcineurin inhibitors (CNI) are being used for the prevention of GvHD, tolerance induction might be hampered due to deteriorated function of regulatory T cells (Treg). Additional data suggest that inhibition of the mammalian target of rapamycin results in suppressed T cell alloreactivity and in sustained Treg function. To explore the efficacy of CNI-free GvHD prophylaxis regimen, we initiated a phase I/II trial using everolimus and mycophenolate-sodium (MMF-Na) as GvHD prophylaxis in HCT pts (EM group, n=23) using peripheral stem cell (PBSC) grafts. Five additional CR1 pts received EM+alemtuzumab (10 mg, EMA group). The diagnoses were: AML/MDS (n=16), ALL (n=3), CML/MPS (n=3), T-PLL (n=1), NHL/CLL (n=6), median age: 49,2 years. 22/28(78%) patients were at high risk (> CR1/CP, untreated) for early relapse. Conditioning included fludarabin based reduced intensity (n=24) or standard regimens containing busulfan (n=2) or clofarabine (n=2). PBSC grafts were obtained from unrelated (n=20) and related (n=8) HLA-matched donors. No graft failure occurred, 20/26 pts showed complete donor chimerism on day +30. T cell reconstitution on day +30 was: CD4+: EM 251,0±101,4, EMA 7,0±14,2; CD8+: EM 175,0±513,9, EMA 6,0±1,9. No grade IV/V toxicities were observed due to the study medication. After a median follow up of 16,2 months, 4 relapses (16,7%) occurred and 9 pts (32,1%) have died. The causes were: progressive disease (n=2), GvHD (n=2), viral infections (n=3), post-surgery thrombembolism (n=1), and one unknown. Treatment related mortality after 1 year was 21,4%. Early recovery of T cell immunity correlated with mild skin GvHD, acute GvHD Grade III-IV could be observed in 7/26 pts. Chronic GvHD occurred in 16/22 pts (moderate n=8, severe n=3). In the first year after HCT no severe bacterial or fungal infections were observed even in cases with prolonged everolimus treatment. The median overall survival was 21 months (EM group) or not reached (EMA group). In conclusion, GvHD prophylaxis with everolimus and MMF-Na is feasible but results in an increased frequency of moderate chronic GvHD without major bacterial and fungal infections. Since this sustained alloreactivity might reduce the risk of relapse this regimen could be suited for patients undergoing HCT with advanced or uncontrolled malignant disease.

P463
Outcome and risk factors for bronchiolitis obliterans following allogeneic stem cell transplantation
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Background: Bronchiolitis Obliterans (BO) is considered a manifestation of chronic graft-versus-host disease (GvHD) affecting 2-20% of patients receiving allogeneic stem cell transplantation (SCT). T-cell mediated allorecognition of lung tissue-antigens is thought to constitute a central basic event, but the pathogenesis is otherwise incompletely understood. Lung biopsy is considered a mainstay in the diagnosis.
Our aim was to investigate the prognostic value of histopathology in lung biopsies in patients suspected for BO and to identify patient-, donor- and transplant related factors related to BO.
Methods: Retrospective analysis of Danish SCT-patients transplanted between 2000 and 2010, who had BO verified by lung biopsy (n=21). All Danish SCT-patients without biopsy-verified BO from the same period (n=741) were used as controls.
Results: Twenty-one patients with histological manifestations of BO were included. Median time from SCT to biopsy was 406 days (range 99-1127). BO was associated with Busulfan based conditioning (40% vs. 20%, p=0,042), while total body irradiation was less common (60% vs. 80%, p=0,047). More BO-patients than controls had a Karnofsky score ≥90 before SCT (90% vs. 60% p=0,0049). CMV seropositivity tended to be more common in the BO group (p=0.055). Overall survival in the BO group did not differ significantly from the controls. Parameters evaluated but not found significantly related to BO, included patient and donor age, sex, presence and grade of acute GvHD, time from diagnosis to SCT, malignant vs. non-malignant diagnosis, donor match, graft type and celldosis.
Conclusion: Conditioning with Busulfan was associated with increased risk of BO, while Karnofsky score was significantly higher in transplants complicated by BO. These findings could indicate that trauma leading to BO primarily occurs during SCT. Busulfan toxicity may increase lung-tissue vulnerability. This is in line with the notion that development of GvHD depends on inflammatory activation of the tissues in addition to T cell mediated alloreactivity towards specific antigens. The fact that overall survival in BO patients was similar to that of the controls may indicate an increased therapeutic focus as well as significant efficiency of the given treatment once a diagnosis of BO has been established.

P464
Experience with extracorporeal photopheresis for treatment of acute steroid refractory graft-versus-host disease
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Steroid refractory acute graft versus host disease (GvHD) following hematopoietic cell transplantation is accompanied by high morbidity and mortality. Up to now there is no established second-line treatment for this group of patients. Extracorporeal photopheresis (ECP) is an extracorporeal photochemotherapy resulting in immunomodulation and has shown benefit in the treatment of severe chronic GvHD.
We retrospectively analyzed 50 patients with steroid refractory acute GvHD treated by ECP at the Ludwig-Maximilians-University

hospital munich between 2006 and 2010, 30 of them have been previously reported in 2009. Grade of acute GvHD was II in 12 patients (24%), III in 21 patients (42%) and IV in 17 patients (34%). Onset of acute GvHD was in median 19 days after transplantation. Refractoriness to corticosteroids was defined as progression or persistence of GvHD after treatment with prednisolone 1-2 mg/kg every 8 hours over a period of 3 days. Patients were treated twice weekly until clinical response, every second week thereafter. Complete response (CR) was defined as discontinuation of corticosteroid therapy following ECP, partial response (PR) as reduction to a maximum of 10 mg per day and minor response (MR) was reduction by 50%. No possibility of steroid reduction or a reduction less than 50% of the initial dose at ECP start judged as no response (NR). After 15 ECP treatments (median) over 64 days (range 8-227 days) 13 patients (26%) obtained CR, 20 PR (40%), 9 MR (18%) and 8 patients (16%) showed no response (NR). Overall survival (OS) during a follow-up time of 1073 days (median 433 days) was 46% (23 patients) with a median OS of 300 days. Patients with acute GvHD grade II (OS 67%) and III (OS 57%) survived significantly better than grade IV (OS 18%), p=0,04. Patients with onset of GvHD before day 19 after transplantation showed significantly poorer survival (32%) compared to patients with late onset (60%), p=0,008. The number of organs, which were affected by GvHD, also had significant influence on survival: 69% in patients with one or two organ GvHD compared to 14% (3 or more organs involved), p<0,001.
ECP may improve OS and clinical outcome in patients with steroid resistant acute GvHD. Further studies are needed to characterize patient subgroups that profit most from this well tolerable therapeutic option.

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Differential induction of IDO and the kynurenine pathway by pre-transplant serotherapy

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Objective: Polyclonal (ATG Fresenius(R), thymoglobuline(R)) and monoclonal (alemtuzumab) T cell antibodies are used for in vivo T cell depletion prior to allogeneic SCT. Differential immunological effects have been postulated. To assess the potential activation of indol-amine-2,3-dioxygenase (IDO) in the course of pretransplant conditioning and serotherapy we measured serum and urine levels of tryptophan and selected kynurenines.
Patients and methods: 40 patients receiving either related or unrelated donor SCT were included. Serum and urine samples were collected prior to conditioning and on d0. All patients gave written informed consent. Tryptophan (Trp) and its catabolites kynurenine (KYN) and quinolinic acid (QA) were measured by liquid chromatography - triple quadrupole mass spectrometry and KYN/Trp ratios were calculated as an indicator of IDO activity.
Results: Mean levels of urine and serum metabolites increased at day -1 or 0. In urine, QA rose from 5.2 (0.7, mean±SE) to 7.0 (1.4) nmol mmol-1 creatinine (ns), KYN from 1.0 (0.1) to 4.9 (0.8) (p<0.001), and the KYN/Trp ratio from 0.13 (0.02) to 0.33 (0.07) (p=0.006); serum levels showed comparable increases, e.g. from 2.9 (0.16) to 3.7 (0.39) for KYN (p=0.05) and from 0.05 (0.01) to 0.07 (0.01) for KYN/Trp (p=0.05). The increases were exclusively explained by the use of polyclonal ATG, for urinary KYN (UKYN) this response was dose-dependent: 10 pts did not receive any serotherapy and d0 UKYN was 1.9 (0.6). 17 Pts receiving 10 mg/kg ATG F had mean UKYN levels of 4.2 (0.6; p=0.02), and 11 pts receiving 20 mg/kg ATG F (n=10) or thymoglobuline (n=1) had mean levels of 8.8 (2.2; p=002 vs ATGF 10 mg/kg). In contrast, 2 pts treated with alemtuzumab showed only a moderate increase in UKYN to 3.3 (0.1; ns vs untreated pts). In multivariate analysis, neither conditioning nor underlying disease had a significant impact, whereas use of polyclonal ATG was the only significant factor (OR 13.3 (95% CI 1.4-126, p=0.02).
Conclusion: Our data demonstrate activation of the kynurenine pathway by serotherapy using polyclonal anti-thmyocyte globulines (ATGs). An IFNγ release induced by ATG might in turn activate IDO. Tryptophan catabolites exert profound suppressive effects on T cell, thus our observation reveals a possible immunosuppressive mechanism of ATGs. As IDO is strongly involved in induction of regulatory T cells, the differential effects of ATGs vs alemtuzumab on T regs might be explained by our observation.

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Xerostomy after haematopoietic stem cell transplantation: an immunohistochemical and morphometric study of minor salivary glands

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Background: Chronic graft versus host disease (cGVHD) is an alloimmune inflammatory process, which results from a donor-origin cellular response against host tissues. 72-83% of patients treated with hematopoietic stem cell transplantation (HSCT) present oral cGVHD. Both, prior HSCT conditioning regimen and cGVHD could cause salivary gland damage and inflammation, leading to xerostomia. Then, significant oral complications develop. T-cells are critical for the induction of cGVHD.
Objective: to compare mucous producing relative acinar area, fibrous relative acinar area and mononuclear cells subsets in minor salivary glands (MSG) of normal individuals and HSCT patients with- and without cGVHD.
Results: 59 patients treated by conventional HSCT entered the study (36 with- and 23 without oral cGVHD). Control group consisted of 19 MSG from normal individuals. MSG biopsies were taken at clinical diagnosis of cGVHD after HSCT. Histological sections were studied on H&E, PAS and Masson's Tricromy (for measurements of relative acinar area), LCA, CD45RO, CD4, and CD8 staining. Digital images were analyzed through Imagelab and LimiarK software. Significant differences for all immunomarkers were found comparing the 2 groups of HSCT patients. But, no differences were found between control group and non- cGVHD patients for the same markers. In the same way, Masson's Tricromy+ acinar area showed significant differences when were compared the 2 groups of HSCT patients, but no differences were found between control group and non- cGVHD patients. PAS+ acinar area exhibited significant differences when the 3 groups were compared each other.
Conclusion: Lymphocyte number and fibrosis in MSG of non-cGVHD patients was shown to be similar to that in control group. So, functional acinar loss is probably the result of conditioning chemotoxicity while fibrous is the later result of inflammatory activity of cGVHD. These finding may be the cause of long standing xerostomia that is observed even in patients who did not develop cGVHD, and also the reason why it is observed persistence or worsening of xerostomia in patients with cGVHD late.
Support: FAPESP.

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A histological study of cGvHD in liver

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Background: Hepatic cGVHD can present as elevation of serum alanine aminotransferase (ALT), alkaline phosphatase (AF), and γ glutamyl transferase as well as slowly progressive cholestatic jaundice and acute hepatocellular injury. According

to the NIH Consensus, histological similarities between acute and chronic liver GVHD do not allow to have a definitive diagnosis of liver cGVHD when no other organ has a diagnostic or distinctive sign of cGVHD.
Aim: to analyze mononuclear cells subsets in liver samples of cGVHD patients, diagnosed by the traditional classification, and correlate it with the NIH histological criteria for liver GVHD.
Methods: samples of liver biopsies of 36 patients who underwent to an identical sibling myeloablative allogeneic hematopoietic stem cell transplant (HSCT) were studied. The biopsies were taken when patients presented sign or symptoms of liver cGVHD. Concomitant infection or medication was ruled out. Biopsy sections were stained for H&E and for immunohistochemical technique targeting LCA, CD4, CD8 and CD138. The samples were blindly and independently analyzed by two observers, based on the NIH criteria for liver cGVHD and compared with liver biopsies taken from non-HSCT patients. Digital images were analyzed through Imagelab software for cell quantification. Statistical study was done by Spearman's correlation and paired t-test.
Results: showed an increased number of immunomarked cells was seen in cGVHD liver, but CD138. Positive correlation was found between liver CD4+ (r=85%; p< 0.0001), CD8+ (r=60%; p=0.002) e CD45+ (r=46%; p= 0.01) cells number/mm2 which confirmed the cGVHD diagnosis. Also, degenerative changes in the ductal epithelium (r=53; p=0.001), bile ducts infiltrated by lymphocytes (r=48; p=0.003), bile duct loss (r=35; p=0.03), portal inflammation (r=54; p=0.001), periportal inflammation (r=57; p< 0.0001) were correlated with cGVHD.
Conclusion: those NIH criteria were confirmed as correlated with liver cGVHD diagnosis.
Support: FAPESP.

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Increased expression of the chemokines CXCL10 and CXCL11 are associated with the development of graft-versus-host disease

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Background: Graft versus host disease (GVHD) remains one of the major causes of morbidity and mortality in HSCT (Haematopoetic stem cell transplantation). Cytokines and chemokines and their receptors play a pivotal role in all phases of GVHD. These molecules provide an important signalling pathway for activation and recruitment of effector cells into sites of inflammation an have been implicated in the rejection of allogenic transplants. Three such chemokines, which have been reported to play a role in transplant rejection are CXCR9, CXCR10 and CXCR11. These three chemokines share a single receptor, CXCR3, which is expressed by T lymphocytes and NK cells. They are inducible by IFN-α and IFN-γ mediated signals and are expressed at sites of inflammation. They attract and activate cells bearing the CXCR3 receptor which results in trafficking and proliferation of T lymphocytes and NK cells cells at the site of inflammation. Recent studies have reported increased serum CXCL10 levels and increased epidermal expression in aGVHD skin biopsies.
Methods: We have investigated CXCL10 and CXCL11 gene expression in normal skin samples, clinical aGVHD skin samples and in a skin explant model of GVHD by real time PCR and Immunohistochemistry. The levels of these chemokines were also analysed in the sera of GVHD patients pre and post transplant.
Results: CXCL10 and CXCL11 gene expression was significantly increased in acute GVHD skin samples in comparison to normal skin samples, p<0.012 for CXCL10 and p<0.003 for CXCL11. Additionally in the skin explant model GVHR positive samples had significantly higher expression by immunohistochemistry for CXCL10 and CXCL11 (p=0.007 and p<0.01, respectively). Similar results were observed with acute GVHD positive clinical biopsies. We observed significantly increased levels of CXCL10 and CXCL11 in acute GVHD patient sera (CXCL10 day 14 p<0.0003 and day 28 p<0.0001, CXCL11 day 14 p<0.001 and day 28 p<0.003) and in chronic GVHD patient sera (CXCL10 6 months p<0.02, CXCL11 6 months p<0.02 and 12 months p<0.0001) compared to controls.
Conclusions: The results indicate a prominent role of CXCL10 and CXCL11 in the pathogenesis of GVHD.

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Development of a population-based cost-effectiveness model of chronic graft-versus-host disease in Spain

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Objective: Chronic graft-versus-host disease (cGVHD) is the leading cause of late non-relapse mortality after hematopoietic stem cell transplantation (HSCT). Chronic GVHD also has a deleterious effect on quality of life in surviving patients who are cured of their underlying disease. Immunosuppressive drugs such as mycophenolate mofetil (MMF) and rapamycin are frequently used as second line treatment (after corticosteroids and calcineurin inhibitors), whereas new strategies such as Rituximab (Rmb), Imatinib (Imt) and Extra-corporeal photopheresis (ECP) are being evaluated as third line treatment.
The purpose of the study was to develop a population-based simulation model of cGVHD in Spain and to quantify the potential health and economic impact of ECP compared to Rmb or Imt, all of them in addition to the usual approach for cGvHD not resolved with prior treatment.
Methods: The model assessed the incremental cost-effectiveness ratio (ICER) and the incremental cost-utility ratio (ICUR) of ECP versus Rmb or Imt for 1,000 hypotetical patients. Microsimulation is a discrete simulation technique which represents single individuals behaviour in a complex system (multiorganic failure). Model probabilities were obtained from literature and

Table 1. Efficacy of ECP, Rituximab and Imatinib. Literature review results.

	Skin	Mucosal	Lung	Liver	GI
ECP	(n: 723)	(n: 256)	(n: 128)	(n: 261)	(n: 70)
Complete R	42%	47%	25%	42%	23%
Partial R	27%	9%	14%	16%	9%
Stable disease	9%	1%	1%	0%	0%
Progression	23%	43%	60%	42%	69%
Rmb	(n: 167)	(n: 44)	(n: 10)	(n: 30)	(n:0)
Complete R	41%	18%	0%	3%	0%
Partial R	35%	30%	30%	27%	0%
Stable disease	4%	9%	20%	10%	0%
Progression	20%	43%	50%	60%	0%
Imt	(n: 58)	(n: 20)	(n: 31)	(n: 1)	(n: 10)
Complete R	17%	5%	13%	0%	20%
Partial R	43%	25%	39%	0%	40%
Stable disease	7%	0%	10%	0%	30%
Progression	33%	70%	39%	100%	10%

Table 2. Cost per improvement gained, Cost per life year gained and cost per Quality Adjusted Life Year gained at 1, 3 and 5 years (ECP vs. alternative)

	COST cumulative	COST difference	Improv.	Improv. gained	Cost per Improv.	LY	LY gained	ICER	QALYs	QALYs gained	ICUR
1 year											
ECP	59,536.33 €		64.8%			0.94			0.75		
Rmb	69,671.42 €	-10,135.09 €	57.6%	7.2%	Dominant	0.93	0.011	Dominant	0.73	0.017	Dominant
Imt	73,813.95 €	-14,277.62 €	62.5%	12.3%	Dominant	0.92	0.024	Dominant	0.71	0.033	Dominant
3 years											
ECP	72,129.48 €		82.0%			2.69			2.20		
Rmb	84,867.61 €	-12,738.13 €	78.4%	3.6%	Dominant	2.65	0.047	Dominant	2.15	0.049	Dominant
Imt	88,329.95 €	-16,200.47 €	77.1%	4.9%	Dominant	2.60	0.091	Dominant	2.11	0.090	Dominant
5 years											
ECP	81,476.83 €		84.7%			4.35			3.59		
Rmb	94,420.35 €	-12,943.52 €	82.5%	2.2%	Dominant	4.26	0.092	Dominant	3.50	0.087	Dominant
Imt	97,687.47 €	-16,210.64 €	81.8%	2.9%	Dominant	4.20	0.145	Dominant	3.45	0.134	Dominant

Improv.: Improvement (% of complete or partial response); LY: Life Year; QALY: Quality Adjusted Life Year; ICER: incremental cost-effectiveness ratio; ICUR: incremental cost-utility ratio

internet search of relevant medical databases (e.g. PUBMED, MEDLINE, CINAHL, DARE, NHS EED, HTA) as well as a targeted search of relevant professional bodies Treatment pathways and adverse events where derived from expert opinion. Local data on health resources use and costs were used and validated by clinical experts. The time horizon of the study was 5 years and only direct local medical costs (in euros 2010) were considered.
Results: Results show that the acquisition cost of ECP is already compensated at 6 months due to its higher efficacy. Improvement differences of 7.2% and of 12.3%, respectively, for Rmb and Imt are shown at first year. The higher efficacy of ECP leads to a gain of 0.017–0.033 Quality Adjusted Life Year at first year and 0.09-0.13 at year 5 when compared to Rmb or Imt. The cost of ECP is lower than Rmb (10,000-13,000€) and Imt (14,000-16,000€). After 6 months, ECP was dominant versus Rmb and Imt for all scores.
Conclusions: Results from this study show that using ECP as third-line therapy for cGvHD results in a dominant strategy (cheaper and more efficacious) at five years with respect to using Rmb or Imt.

P470
IL-17 and CCR6 positive cells are present in the skin and gut with toxic lesions and even more pronounced in aGvHD
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Objectives: Graft versus host disease (GvHD) is a multistep inflammatory process triggered by allogeneic activation of donor T cells in the skin and intestine. Biologically is described as a cytokine storm. More recently Th17/CD4+ cells were shown to be active in inflammatory bowel disease as a pro-inflammatory cytokine. Therefore, we performed a study on the presence of Il-17+ and CCR6+ cells in the affected tissues in patients after hematopoietic stem cell transplantation (HSCT).
Methods: 28 specimens (16 skin and 12 digestive tract biopsies) obtained from 18 patients (3 with toxicity and 15 with aGvHD post HSCT) were assessed immunopathomorphologically for the presence of T cells (CD3, CD4, CD8), activation marker DR and for the presence of IL-17 and CCR6 (paraffin block sections). Moreover, the tissue infiltrating cells were isolated from the biopsied tissues (after enzyme digestion) stained and read by cytometry for profiling, and assessed for mRNA IL17 gene expression.
Results: IL-17+ cells were present in all tissue sections in areas with lymphocyte infiltrations, however, in the skin were rather scarce and no more than 1 or 2 IL-17+ cells were present in 3 high power fields (HPF: 400x). CCR6 were more frequent, and characteristic granular staining was seen in more than 10 cells per 1 microscopic HPF. Moreover, CCR6 positivity was clearly seen in keratinocytes, especially in areas with aberrant expression of DR antigens.
In the gut the contribution of IL-17 and CCR6 positive cells was greater than in the skin, and IL-17 positive cells were in 10-20% of all mononuclear cells. CCR6 was present on infiltrating cells and also in some crypt epithelial cells. Six digestive tract biopsies with toxic lesions were less infiltrated with inflammatory and IL-17+ cells. However, CCR6 positivity on the crypt epithelium was much stronger than in the aGvHD lesions. In all these cases single cell suspension analysis confirmed the presence of CCR6 positive cells in CD45 negative cell compartment. At the mRNA level in all 9 investigated specimens a high level of IL-17mRNA was seen.
Conclusions: IL-17 and CCR6 positive mononuclear cells contribute to the cell infiltration in both toxic and aGvHD lesions in patients post-HSCT. However, these cells are more associated with aGvHD than with post-transplant toxicity and were in the highest frequency when the digestive tract was a target organ.

P471
Epithelial expression of CCR6 chemokine receptor and the presence of IL-17 producing cells is associated with skin pathology in chronic GvHD
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Objectives: Chronic graft-versus-host-disease (cGvHD) is a distinct clinical and pathological entity as a late complication after hematopoietic stem cell transplantation (HSCT). In this study we have evaluated the expression of CCR6 in the context with the presence IL-17 cytokine phenotypes T-cells and regulatory T-cells FoxP3 in the course of cGvHD.
Methods: A total of 12 specimens were harvested from 9 patients with clinical symptoms of cGvHD after HSCT (5 from matched unrelated donors and 4 from sibling donors). Twelve biopsies: 7 skin biopsies, 3 oral mucosa and 1 specimen from stomach mucosa and gut epithelium were analyzed immunopathomorphologically for the presence of tissue infiltrates (CD3, CD4, CD8), DR expression and for the presence of IL-17 T-cells, FOXP3 cells, and CCR6 expression. The tissue infiltrating cells were isolated from the damaged section after tissue digestion and assessed for the presence of CCR6+/CD45+ and CCR6+/CD45- cells by flow cytometry.
Results: In the group of cGvHD patients tissue infiltrating cells were composed from CD4+/DR+ cells. Moderate number of T-cells expressing IL-17 were identified in all skin biopsies. FOXP3 positive cells were found in 2/7 skin biopsies only. Strong expression of CCR6 was seen on keatinocytes and infiltrating cells in the skin.
In oral mucosa the number of IL-17 cells was more pronounced and was higher or similar to the cells expressing CCR6. Numerous FOXP3 positive cells were present in 1/3 oral mucosa only. In contrast to the skin, epithelial cells of oral mucosa did not express CCR6 and the number of infiltrating cells expressing CCR6 was seen in 1/3 specimens of the mucosa.
FC confirmed that CCR6+/CD45- cells are in prevalence over CCR6+/CD45+ cells in the skin but not in the oral mucosa.
Conclusions: Th17 lymphocytes constitute a hallmark of lymphocyte infiltration in cGvHD. CCR6 positive cells are common in the skin but not so frequent in mucous lesions. Epithelial cells are frequently CCR6 positive in the inflamed areas especially in the skin. FoxP3 are present only in a proportion of cases. Therefore, Th17 cells are rather poorly controlled by Treg cells in cGvHD tissue lesions.

P472
IL-17, IFN-γ and FoxP3 positive cells in blood in patients post alloHSCT
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Alloreactivity strongly influences the course post HSCT being a primary force in pathomechanism of aGvHD, shapes the immunological reconstitution post transplant and mounts graft vs leukemia effect. Therefore the undestanding of the interplay between the main subsets of CD4 positive cells may help in understading the mechanism which facilitates the activity of the immune system post HSCT. For that we investigated the presence of cells with a potential to generate IL-17, IFN-γ and FoxP3 in the first wave of lymphocytes appearing in blood post HSCT. PBMC were purified from blood of 64 pts (median age: 42 yrs, range; 1.0-64 yrs) taken at the time of hematological recovery (time post transplantation: median:14.5 days, range: 7-31 days). Fourteen of them developed aGvHD at that time.

PBMC were stimulated with Brefeldin A, Ionomycin and PMA and then labeled for CD4 (BD, San Jose, CA), intracellular IL-17A and FoxP3 (e-biosciences, San Diego, CA) and IFNγ (BD) detection.
We found:
1) Pts post alloHSCT had higher proportions of IL-17A producing cells post HSCT as compared to healthy donors (0.73±0.12 vs 0.19%±0.06, p=0.03).
2) There is a significant correlation between the contribution of IL-17A and IFNγ producing CD4 lymphocytes at the time of hematological recovery in all pts group (r=0.29, p=0,07.) and also in a subgroup of pts with skin aGvHD (r=0.81, p=0.015).
3) Pts with apparent skin symptoms of aGvHD manifested early post HSCT had lower proportions of both IL-17A (0.29%±0.09 vs 0.73%±0.12, p=0.03, M-W U-Test) and IFNγ (0.08%±0.26 vs 0.57%±0.99, p=0.16, M-W U-Test) in blood as compared to counter-partners lacking aGvHD.
4) In pts with a high proportion of IL-17A producing cells in CD4 lymphocytes there was 71% of pts with a high proportion of FoxP3+ CD4 lymphocytes and only 41% in pts with low proportions of IL-17A producing cells in CD4 lymphocytes (p=0.04, Fisher exact test).
5) Proportions of INFγ producing cells were correlated with proportions of FoxP3 producing cells in a group of pts with skin aGvHD (r=0.81, p=0.013), but not in pts lacking aGvHD at the same time post transplant.

Correlations found between IL+17A, IFNγ and FoxP3 producing cells represent a regulatory network operating post HSCT, Supported by the grant N N402 430039 from the Polish Ministry of Science & Higher Education.

P473

Mild pre-engraftment immune reaction may be associated with better survival and GVL effects with less TRM after cord blood transplantation for haematological malignancies

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Background: We previously reported early immune reaction named pre-engraftment immune reactions (PIR) after reduced-intensity cord blood transplantation[1,2]. Recently, although the similar phenomena named pre-engraftment syndrome(PES) after CBT were reported, the clinical impact about survival and treatment related mortality (TRM) was controversial.
Methods: To estimate the effect of PIR, we defined PIR severity, mild type as the series of symptoms consist of fever, skin rash, weight gain, diarrhea and peripheral edema 6 within grade 2 of acute graft-versus-host disease(aGVHD)or more days before engraftment, and severe type as PIR with organ dysfunction (lung, kidney, and HPS). We retrospectively analyzed of 355 patients who had received first CBT at Toranomon Hospital, Tokyo, Japan.
Results: Most of them had diseases in advanced status (77.2%). The pre-transplant conditioning were mainly consist of reduced intensity (n=307). GVHD prophylaxis were calcinurin alone (n=246) and Tac plus MMF(n=109). HLA antigen (A,B and DR) disparities were mainly 4/6 matched (70.1%). Underlying diseases were myeloid malignancies (n=194), lymphoid malignancies (n=146) and SAA (n=13).
The Median observation period after CBT was 1314 days (range, 140-2898). Survival (OS and EFS) of 3-years were 32.9% (27.8-38.0) and 27.2% (22.5-32.0). The incidence of PIR was 63.8% (mild: 35.7%, severe: 27.9%). The cumulative incidences of aGVHD grade II-IV and chronic GVHD (cGVHD) were 52.3% and 49.0%, respectively. In multivariate analysis, PIR was associated with high TRM (RR1.94), low relapse rate (RR1.72), frequent aGVHD (RR1.98) and cGVHD (RR2.79), not but better OS and EFS. Severe PIR affected on poor survival. Interestingly, in subgroup analysis based on disease type, mild PIR affected better EFS than no or severe PIR in both lymphoid (p=0.007) and myeloid (p=0.042) malignancies.
Conclusions: Although severe PIR result on high TRM and poor survival, mild PIR play a role on lower relapse rate and better survival in high risk hematological malignancies without a/c GVHD. Further observations about the GVL effect were needed to evaluate PIR.

P474

GvHD post allogeneic transplantation: frequent readmissions and high costs

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Graft-versus-host disease (GvHD) remains a common complication of haematopoietic stem cell transplant (HSCT) but little is known about the rate of hospital readmissions and costs associated with the disease. 196 patients underwent HSCT at the Royal Marsden Hospital between 1/1/2006 and 31/3/2009. 96/196 (49%) developed GvHD. Median follow up was 2.5 yrs (1-4 yrs) and the number of readmissions and associated costs were assessed. Patient characteristics: median age 46.5 (17-70) yrs; male 57; diagnosis: acute leukaemia 60, lymphoma 9, chronic leukaemia 13, myeloma 7, other 7. Transplant type: unrelated donor 63, sibling 28, cord 5; peripheral blood 85, bone marrow 6; prior allogeneic transplant 5. Median cell dose: $5.72x10^6$ CD34+cells (0.03-12.37). Conditioning: full intensity 39, reduced intensity 57, in vivo Alemtuzumab 52. GvHD prophylaxis: ciclosporin (CSA) 78, CSA + methotrexate 9, CSA and mycophenolate mofetil (MMF) 5, MMF 4. 43 patients developed

Table 1: Patient Characteristics

Patient Characteristic	No of Patients (n=96)
Median Age: 46.5 years (17-70)	
Male	57
Female	39
Disease	
Acute Leukaemia	60
Lymphoma	9
Chronic Leukaemia	13
Myeloma	7
Other haematological malignancies	7
Type of Transplant	
Volunteer Unrelated Donor	63
Sibling Allogeneic	28
Umbilical Cord	5
Previous Allogeneic Transplant	5
Donor Lymphocyte Infusion	8
Source of Stem Cells	
Peripheral Blood	85
Bone Marrow	6
Cord Blood	5
Median Cell Dose 5.72×10^6 CD34$^+$ cells (0.03-12.37)	
Donor Sex	
Male	59
Female	32
Cord Blood	5
Conditioning	
Full Intensity	39
Reduced Intensity	57
In vivo Alemtuzumab	52
GvHD Prophylaxis	
Ciclosporin	78
Ciclosporin and Methotrexate	9
Ciclosporin and Mycophenolate mofetil	5
Mycophenolate mofetil	4

acute and 14 chronic GvHD, 31 had both acute and chronic disease and 8 developed GvHD post donor lymphocyte infusion. In those with acute GvHD, 14 had grade 1 disease, 35 had grade 2, 12 had grade 3 and 13 had grade 4. 90/96 (94%) received steroid treatment. 49/96 (51%) were steroid refractory. The median duration of transplant admission was 31 days (20-138). 5 patients died during initial admission. The total number of readmission episodes was 266 with a readmission rate of 79/91 (87%). The median number of readmission episodes was 2 (0 to 12). The median time to first readmission was 57 (22-518) days from transplant and 26 (1-498) days from discharge from initial transplant admission. 33% of readmissions occurred within 100 days of transplant and 83% occurred within one year. The median number of readmission days was 30 per patient (0-216). 30 (31%) patients required critical care unit (CCU) admission. The median CCU stay was 6 days (1-34). Costs were calculated based on the 2010 hospital tariff (Euro600/inpatient day and Euro2295/CCU day). The median cost of readmission per patient was Euro20400 (0-131295) with an approximate total cost of Euro3 million. This figure did not include additional medication or procedural costs. Overall survival was 43 % at 2 years. Causes of death: infection 19/96 (20%), GvHD 12/96 (12%), relapse 17/96 (18%) and other 3/96 (3%). This study shows the high readmission rates and cost associated with GvHD and highlights the need for new treatment approaches for this condition.

Table 1: Patient Characteristics

CHARACTERISTIC	No. (%) n=82
Male	50 (61)
Female	32 (39)
Median age (range) years	44.7 (14.1-69.5)
Diagnosis:	
Acute leukaemia/Myelodysplastic Syndrome	33 (40)
Lymphoma	18 (22)
Chronic Lymphocytic Leukaemia	6 (7)
Chronic Myeloid Leukaemia	10 (12)
Myeloma	8 (10)
Other	7 (9)
Donor type:	
Unrelated donor	23 (28)
Sibling/Related donor	59 (72)
No of immunosuppressive agents at start of ECP	
None	11 (14)
One	10 (12)
Two	40 (49)
Three	13 (16)
Four or more	6 (7)
Unknown	2 (2)
No of organs involved by cGVHD	
One	35 (42)
Two	31 (38)
Three	12 (15)
Four or more	4 (5)

P475

Clinical improvement and reduction in immunosuppression following extracorporeal photopheresis for chronic graft-versus-host disease

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We report on 82 patients who commenced extracorporeal photopheresis (ECP) treatment for chronic graft versus host disease (cGVHD) from 01/01/2005 to 1/03/2010. Patients initially received a bimonthly regimen of 2 ECP treatments on consecutive days (1 cycle) which was subsequently tapered to a monthly regimen depending on response. Immunosuppression was tapered by the referring centre. Data were collected from the unit's GvHD database. Patient characteristics are in table 1. The median time from transplant to ECP was 2 yrs (0.5-10). All had mucocutaneous disease. 75 had skin disease (40 sclerodermoid, 34 lichenoid, 1 both). 39 had oral disease. All patients were steroid refractory or intolerant and 72% were on 2 immunosuppressive drugs prior to starting ECP. 69 were on immunosuppression (prednisolone 62 (median dose 25mg (5-135)), ciclosporin 39, mycophenolate mofetil 29, and other 16). 52 had completed ECP at the time of analysis. The median duration of ECP was 330 days (42-987) and the median number of ECP cycles was 15 (1.5-32). The remaining 30 had received at least 6 months of ECP. 50 had reduced the frequency of ECP to monthly. The median time to reduction was 207 days (74-638). Response was assessed after 6 months by a single investigator. Doses of immunosuppression, skin/oral scores and functional assessment were documented. 69/82 patients were assessed (6 died and 7 had completed <6 months ECP). Overall 65/69 (94%) improved. 5/69 (7%) had a complete improvement in their symptoms and signs of GvHD and 60/69 (87%) had a partial improvement. 4 had stable disease. 52/69 patients on immunosuppression were reassessed and 41/52 (79%) had a dose reduction. 40/49 (82%) decreased their steroid dose (11 stopped, 12 had ≥ 75% reduction, 7 ≥ 50% reduction and 10 < 50% reduction). 4 doses were stable and 5 had increased. The median dose was 10 mg (3-50 mg). All patients were included in the survival analysis including those who had completed < 6 months of ECP. Overall survival at 3.2 years from the start of ECP was 69%. 19 patients died. Causes of death: GvHD alone (4), infection (5), infection and GvHD (6), relapse (2), unknown (2). Responders to ECP (a ≥ 50% reduction in steroids or no re-introduction of immunosuppression) had 75% survival at 3.2 years compared to 63% in non-responders (p=0.05). This study confirms that ECP is highly effective in improving symptoms and signs of cGVHD and allowing reduction of immunosuppression.

P476

Catheter-directed intra-arterial organ-specific anti graft-versus- host disease immunosuppressive therapy for patients with steroid resistance or dependence

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Purpose: To prospectively investigate the efficacy of intra-arterial steroid (IAS) injection using for the treatment of steroid resistant or dependent graft-versus-host-disease (GVHD).

Methods: Consecutive patients with steroid resistant or dependent GVHD treated with IAS were enrolled. Patients with hepatic GVHD were treated with a hepatic arterial infusion of 600 mg/m^2 methylprednisolone (MP), (maximal dose of 1000 mg). Patients with gastrointestinal (GI) GVHD were treated with an IA infusion of MP 40-60 mg/vessel into the superior and inferior mesenteric arteries with the addition of 40 mg of MP to each internal iliac artery or more selectively if technically possible. In patients with pronounced upper GI symptoms, 40 mg MP was infused into the gastro-duodenal artery. Patients in whom GVHD was marked in both liver and GI tract had both treated in the same session. Non-parametric tests and confidence intervals were used. Kaplan-Meier survival curves were used to estimate time to death and time to remission.

Results: Fifty-five patients (median age 39.7 years, range 7.6–69 years) with steroid resistant or dependent GVHD (liver – 11, GI – 27, combined liver and GI – 17) underwent IAS within a median time of 3 weeks from GVHD diagnosis. Partial or complete liver and GI response rates were 53.6% and 65.9%, accordingly. The one year survival in our cohort was 36%. 20 patients died within one year of GVHD (36%). Other causes

of death included infection (9), disease progression/relapse (5), others (1). We found a significant association between remission in the IAS treated organ and increased survival (liver and GI remission, p<0.01 and p=0.03, respectively). Previous autologous transplant and TBI were significantly associated with higher 1-year mortality (p=0.036 and 0.0007, respectively). There was no association between age, sex, primary diagnosis, 1 vs. 2 treated organs, donor matching, number of previous transplants, and ablative vs. RIC to survival. Eight patients (14.6%) had renal failure after IAS (mostly transient), that was not associated with increased 1 year mortality.
Conclusion: Data from this largest cohort of IAS reported to date in the English literature suggests that IAS may be a safe and effective means of inducing response in patients with steroid resistant GVHD. Consideration should be given to routinely combining IAS with standard GVHD treatment regimens. Further research is warranted to assess the optimal dosing and administration protocol.

P477

Advances in medical and supportive care significantly improved the survival of patients with severe acute GvHD and changed its natural course

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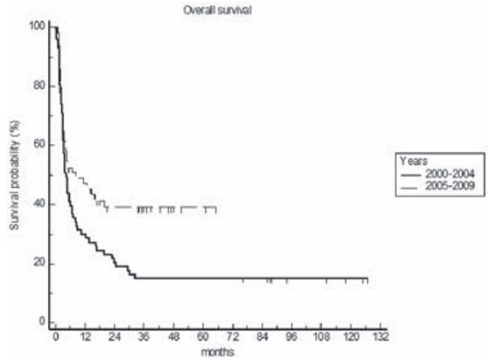
Introduction: GVHD is the most ominous side-effect of allogeneic stem cell transplantation and when refractory to steroids, carries increased morbidity and extremely high mortality rates.
Methods: in the last 5 years we introduced novel techniques and drugs aimed at the treatment of steroid refractory acute GVHD, in parallel to advances in supportive care. These included intra-arterial steroid treatment of affected organs, mesenchymal cells and alefacept, combined with optimal symptom management emphasizing intensive and coordinated multidisciplinary team work. In this study we assessed the effect of these changes in management of severe GVHD on overall survival.
Results: During the period encompassing this study (2000-2009), 554 patients underwent 587 matched allogeneic transplants in our center. Of the 554 patients, 130 suffered from severe (grade 3-4) GVHD (22%). The patients were divided into two time periods: Years 2000-2004 (73 patients) and 2005-2009 (57 patients). GVHD prophylaxis consisted of cyclosporine as a single agent in both groups. The groups did not differ in age, sex, disease type (malignant vs. non malignant) or median time to acute GVHD. There was higher rate of unrelated donor transplantation in the 2005-2009 group with a strong trend toward significance (25/57 vs. 16/73, p=0.08).
Respectively, 30% and 47% of the patients with severe GVHD transplanted in the years 2000-2004 and 2000-2009 survived 1-year from the transplant (figure 1, p=0.018). At the time of this report, 11/73 and 22/57 are alive, mostly disease free, mostly with little or no chronic GVHD.



Conclusions: the introduction of novel techniques and drugs as well as the improvement of supportive care significantly improved the outcome of severe acute GVHD and changed its natural course.

P478

Single dose post-transplant ATG for GvHD prophylaxis might increase GvHD risk

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Introduction: Anti thymocyte globulin (ATG) was introduced to allogeneic stem cell transplantation mainly in order to prevent graft rejection, and thus was given on the last conditioning days. During the last years, it was appreciated the role ATG is more pronounced as GVHD prophylaxis. We therefore assessed the effect of a change in the timing of ATG infusion from pre-transplant days to post transplant day +1.
Methods: Seventeen patients (12 males, 5 females; median age 48 years (12-66)) with hematological malignancies were included. All received fludarabine-busulfex based protocols and were transplanted from full HLA-matched siblings. GVHD prophylaxis consisted of cyclosporine started on day -4 and a single dose of IV thymoglobulin 2.5 mg/kg given on day +1.
Results: All patients engrafted (one after infusion of a second graft) with a median time to recovery of absolute neutrophil count >0.5x10^9/L of 13 days (range 9-23) and platelet recovery >20x10^9/L in the range of 8-24d (median 11d).
Acute GVHD of all grades was seen in 14/17 (82%, figure 1) of the patients, grade 2-4 GVHD was note in 9/17 (53%) and grade 3-4 occurred in 4/17 patient (24%, figure 2). Chronic GVHD was present in 12/13 evaluable patients (limited in 7 and extensive in 5). GVHD associated death occurred in 2 patients.
Conclusions: post transplant infusion of ATG may adversely increase the rate of GVHD, possibly due immune stimulation of donor T-cells by the antibody.

Figure 1

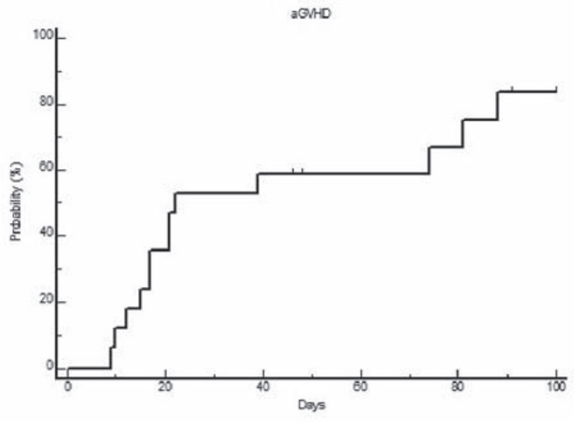
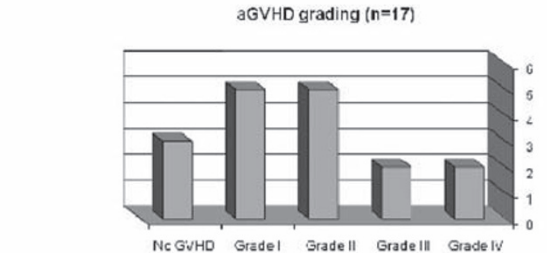


Figure 2



P479

The impact of cGvHD on anti-infectious immune reconstitution after HCT and practical guidelines for vaccination schedules

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The organ involvement in the course of chronic graft versus host disease (cGvHD) can be associated with various forms of secondary immunodeficiencies that might affect anti-infectious immune reconstitution. Revaccination after allogeneic hematopoietic cell transplantation (HCT) is an important part of the post-transplantation care. In order to find factors affecting immune response to vaccination after HCT, a prospective study with vaccination against hepatitis B virus (HBV) was carried out.

42 patients qualified to active immunization against HBV had the immunosuppressive treatment discontinued for at least 2 months. Patients with cGvHD history were included if they did not suffer from the active phase of disease requiring immunosuppressive therapy. Chronic GvHD was diagnosed in 25 (59,5%) patients. According to ASBMT criteria 36% of them suffered from the mild, 28% moderate and 36% severe grade of cGvHD.

Seroconversion after vaccination with recombinant HBs antigen (rHBsAg) was achieved in all patients. Depending on the achieved titer of antiHBs antibodies, total vaccine doses and the maintenance of protective antiHBs level, 12 patients were classified as weak responders (WR), 16 as good responders (GR) and 14 as very good responders (VGR). Statistical analysis showed that the grade of cGvHD has an impact on the immunization: 75% of WR was treated before immunization because of severe cGvHD (p=0,057) even though the immunization was performed in non-active phase of the disease.

While the immunosuppressive agents were no longer administered at the time of immunization, it was cGvHD itself or immune disturbances leading to cGvHD that weaken the response to HBV vaccine. Chronic GvHD is the state of chronic inflammation with significant population of suppressor T lymphocytes able to inhibit B lymphocytes from the production of specific antibodies and interfere in the transformation of Th precursors into mature Th lymphocytes. Enhanced polyclonal activation and immune attrition provoked by chronic antigenic stress could be another explanations. Telomere attrition might be the reasonable mechanism involved.

Since severe cGvHD is the factor weakening efficacy of the active immunization it is essential to choose the right moment of vaccination and to use the double dose of rHBsAg (40µg) until the protective level of antiHBs antibodies is achieved, as well as to monitor the antiHBs titre as re-immunization might be necessary.

P480

Second-line treatment in steroid refractory acute graft-versus-host disease during a 10-year period: a single-centre experience comparing 3 strategies

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Background: Steroid-refractory acute graft-versus-host disease (SR-aGVHD) is a significant cause of mortality after allogeneic hematopoietic stem cell transplantation. There is no consensus concerning the optimal second-line treatment (SLT) for SR-aGVHD. We successively used as SLT calcineurin inhibitor (CNI) + mycophenolate mofetil (MMF) [1999-2004] and etanercept or inolimomab [>2004].

Aim: compare survival of patients (pts) who received the 3 different strategies.

Method: 93 consecutive pts were included. Survival curves were estimated by Kaplan-Meier estimator. SR-aGVHD was defined as progressive disease at 3 days, stable disease at 7 days or partial response at 14 days after steroid initiation. Viral, bacterial and fungal infections were analyzed, death being considered as a competing event. The association between SLT and infection was analyzed using Cox proportional cause-specific hazards model.

Results: Median age was 37 years. Conditioning regimen was myelo-ablative in 65% and donor was unrelated in 68%. Stem cell source was peripheral blood (PBSC) in 44%, bone marrow in 42% and cord blood in 14%. The most used GVHD prophylaxis was ciclosporin with methotrexate or MMF. GVHD occurred at a median of 15 days after transplant. SLT was CNI/MMF in 56%, inolimomab in 22% and etanercept in 23%, and was initiated a median of 12 days after GVHD diagnosis. Grade 3-4 aGVHD was more frequent in pts treated by etanercept (72%) compared to inolimomab (35%) or CNI/MMF (41%). Overall response to SLT was 45% (complete response: 28%): 55% with CNI/MMF, 35% with inolimomab and 28% with etanercept, respectively. With 74 months median follow-up, 2-year survival was 30% (95% CI: 22-41). Risk factors for overall survival in univariate analysis were age (95%CI: 1.01-1.37), disease status at transplant (95%CI: 1.85-5.27), PBSC as graft source (95%CI: 1.07-3.20), recipient CMV positive status (95%CI: 1.07-2.97), grade 3-4 GVHD (95%CI: 1.77-4.86), liver involvement (95%CI: 2.39-6.74) and etanercept as SLT (95%CI: 1.02-3.32). In multivariate analysis, only disease status at transplant, grade 3-4 GVHD and liver involvement were significantly associated with survival. Probabilities of viral and fungal infections were not statistically different among the 3 SLT groups but bacterial infection rate was higher after inolimomab and etanercept.

Conclusion: 3 strategies of treatment including 2 anti-cytokines treatment over a 10-year period give similar survival in pts with SR-aGVHD.

P481

Platelet gel is highly effective in treating muco-cutaneous lesions related to graft-versus-host disease

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Background: Platelet (PLT) gel has been successfully used in tissue regeneration of diabetic/surgical wounds through the releasing of growth factors such as bFGF and PDGF. Therefore, the PLT gel could represent a therapeutic tool in treating the deep and painful wounds occurring during Graft-versus-Host Disease (GVHD).

Objective: The aim of this study was to verify the efficacy and safety of PLT gel for treating muco-cutaneous GVHD ulcers. Allogeneic hemocomponents were used to obtain PLT gel with Vivostat System. Ten patients with multiple lesions involving dermis (grade I, n=2), subcutaneous or oral mucosa (grade II, n=8) related to acute or chronic GVHD, underwent PLT gel as local therapy.

Results: After the second PLT gel application, the pain disappeared in all cases and the granulation tissue was observed in all patients with grade II lesions. After a median of 6.5 PLT gel applications (range, 1-12), 9 of 10 patients riched a complete response, while 1 patient showed a partial response at time of death because of multi-organ failure. No side effect was documented.

Conclusion: These results confirm our previous data mainly related to the treatment of GVHD skin wounds, hampering the use of PLT gel as effective tool also in the management of oral mucosa lesions frequently associated with chronic GVHD.

P482

Treatment of hyperacute graft-versus-host disease with early extracorporal photopheresis and standard dose of methylprednisolone after volunteer unrelated donor allograft

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Hyperacute graft versus host disease (GVHD) - occuring within 14 days after transplantation- is an agressive form of acute GVHD resulting lower response rate to first line therapy and higher rate of non-relapse mortality after allograft. From November, 2009, we treated 8 patients (5 Male, 3 Female median age: 40,5 (25-58)) with hyperacute GVHD among 71 allograft recipients (median follow up period 10,5 (1-13) months) All the 8 patient received the transplant because of high risk hematological malignancy (4 acute myeloid leukemia (AML), 2 acute lymphoid leukemia (ALL), 1 mycosis fungoifdes (MF), 1 Sezary sy.). All of them had volunteer unrelated donor (VUD) graft (2 matched, 5 one antigen mismatched, 1 two antigens mismatched). Five patients received 12 Gy total body irradiation/Cyclophosphamide (TBI/Cy) conditioning, two patients oral 16mg Busulfan/cyclophosphamide (Bu/Cy) conditioning and one Busulfan/Fludarabine/anti-thymocyte globuline (BU/FLU/ATG) reduced intensity conditioning (RIC). Six patients received unmanipulated periferal blood stem cell (PBSC), 2 patients received CD34 positively selected graft. Standard GVHD prophylaxis was sirolimus/tacrolimus combination. Median onset of acute GVHD was day 8,5 (6-14), with severe skin involvement (3 grad II, 4 grad III, 1 grad IV). Median time of engraftment was day 11,5 (7-14). Patients received 2 mg/bw kg methylprednisolone and extracorporal photopheresis (ECP) after the engraftment. The median start of ECP was day 14 (10-27) and median cycle of ECP was 31 (10-44). All patients responded to therapy 6/8 achived complete resolution of acute GVHD, 2/8 patients responded partially. Six patients survived, 2 died of infection (1 Gr- sepsis, 1 Cytomegalovirus (CMV) pneumonitis). Two patients have no chronic GVHD, 2 patients limited, 2 patients extensive chronic GVHD. In conclusion standard dose metyhlprednolone and ECP had strong effect controlling hyperacute GVHD in our patients without the need of second line treatment maintaining enough immun response to survive acute GVHD and its therapy. Further studies are warranted.

P483

Tacrolimus and short courses sirolimus graft-versus-host disease prophylaxis regimen for HLA-identical sibling myeloablative peripheral blood stem cell transplantation

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The purpose of our study to find a good balance between toxicity and efficacy and graft versus tumor effect with tacrolimus and short sirolimus graft versus host disease (GVHD) prophylaxis regimen. We followed the regimen published by the Dana Farber group but we truncated the administration of sirolimus at day 30 post-transplant and maintained tacrolimus until 180 days. From January 2006 to December 2009 we transplanted 46 patients (23 male, 23 female) patients with acute myeloid leukemia (30), acute lymphoid leukemia (6), advanced chronic myeloid leukemia (4), and myelodysplastic syndrome (5). 22/46 patients belonged to the standard risk group and 24/46 to the high risk group. The median age of the patients was 40 (15-61) years. All patients received myeloablative conditioning regimen and unmanipulated peripheral stem cell graft from a HLA identical sibling donor. The median follow up period is 11.9 (1.2-47) months. All patients engrafted, and reached full donor chimerism and complete remission by bone marrow histology at day 30. The cumulative incidence of acute GVHD was 34%, 13 patients had grade I-II, 2 patients grade III and all of them were steroid responsive. 18/46 patients had chronic GVHD (9 limited, 9 extensive). The toxicity was mild, 3 patients had sinusoidal obstructive syndrome (SOS), 4 patients transplant associated microangiopathy (TMA). We observed 1 CMV disease, 1 invasive fungal infection, and 8 bacterial blood stream infection. Eleven patients died (5 relapse, 5 chr. GVHD+infection, 1 secondary malignancy). 44/46 (95%) of the patients lived at day 100, 39/46 (85%) at one year, 35/46 (76%) at two years. The probability of four year survival is 63% for the whole cohort, 87% for the standard risk group and 49% for the high risk group of patient. Our patient cohort is in line with the data published by the Dana Farber group but seems to be less toxic regarding SOS syndrome and TMA.

P484

IL-21 serum levels in allogenic stem cell transplantation are associated with acute graft-versus-host disease

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20'000 patients/year are receiving a allogeneic stem cell transplantation (allo-HSCT) for high risk haematological malignancies or inborn disease worldwide. Despite GvHD prophylaxis 30-60% of patients develop acute graft versus host disease (aGvHD) with wide ranging effects on immune reconstitution, infectious complications, quality of life and ultimatively survival. Recently, we and others have described pre-emptive IL-21 blockade as a promising strategy to decrease aGvHD lethality while allowing for relevant graft-versus-leukemia effect (GvL) in murine models of aGvHD.
Objective: Describe serum levels of IL-21 in the peritransplant period in humans. Test for association of serum-IL-21 levels pre and post transplant with the occurrence of aGvHD.
Methods: Serum samples of 29 consecutive patients undergoing allo-HSCT for AML (n=17), MDS (12) or ALL (6) from a HLA-identical donor after full intensity conditioning (CyBU or Cy/(VP16)/TBI) at from 11-2005 to 11-2007 were included. Data on remission status, CMV-status of recipient and donor, aGvHD, CRP and infectious complications was obtained from primary patient charts and the transplant database. IL-21 serum concenteration was measured by ELISA.
Results: 87 serum samples from 29 patients were obtained. From each patient, samples were taken pre-conditioning, early post-transplant and late post-transplant (median d-9, d+10, d+18). Median IL-21 serum level at the three time points were 3.89ng/ml(0-585), 0(0-1226) and 0(0-1258) respectively. Nine patients had GvHD >II. Median IL-21 serum levels of patients with aGvHD at the three time-points were 181, 70.3 and 226 respectively. A significant association of IL-21 serum levels with aGvHD was found pre-conditioning and late posttransplant (p=0.023 and 0.021respectively) while only a trend (p=0.07) was found early post-transplant.
No association of IL-21 serum levels with the other parameters mentioned above was found in uni-variate analysis. Multivariate analysis was omitted due to the small sample size. These data suggest that IL-21 serum levels pretransplant are associated with the later occurrence of aGvHD and that elevated IL-21 serum levels might reflect GvHD activity.
Conclusion: These preliminary findings suggest that the IL-21 pathway described in experimental models might be relevant in humans and therefore deserve further study.

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Usefulness of wireless capsule endoscopy for the diagnosis and management of gastrointestinal graft-versus-host disease

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Background: The most common location of Gastrointestinal Graft Versus Host Disease (GI-GVHD) is the small bowel (SB), but while jejunum and ileum are not usually accessible by endoscopy, wireless capsule endoscopy (WCE), a non-invasive imaging procedure, offers a complete evaluation. Objective: To establish the usefulness of WCE, in diagnosis and management of GI GVHD.

Patients and methods: Prospective study of 13 patients with clinical suspicion of GI-GVHD in our Unit, between January 2009 and September 2010. Patients underwent both upper and lower endoscopy (UGE/LGE) and WCE.

Results: Median day of onset of symptoms after transplant was +100 (range 14-298). The median follow-up was 241 days (range 46-586). 7 patients presented as stages 1-2 GI-GVHD (mild GVHD) and 6 as stage 3-4 (severe GVHD). 6 patients in the mild GVHD group reached complete remission (CR) after 1st line therapy, 1 patient needed a 2nd line. On the severe GVHD group, only 1 patient reached CR after 1st line therapy. UGE/LGE was performed on the day of onset in most cases. WCE was performed at a mean day after onset of +15 (range 0-75), in 1 patient the WCE was performed 75 days after onset because of persistence of upper GI symptoms with previous negative screening. On the mild GI-GVHD group, the UGE was normal in 2 cases, showed erythema in 3 and focal erosions in 2. GvHD was confirmed by histopathologic studies in 5. The WCE was normal in 1 case, showed ulcers in SB compatible with GI GVHD in 4 and in 2 delayed gastric transit time (DGTT) was the only finding. In one patient with normal UGE and LGE, the capsule confirmed the GVHD. In the severe GVHD group, all the LGE performed were normal, whereas UGE were normal in 2 cases, showed erythema in 1 and focal ulcers in 3. The histopathologic studies confirmed GVHD in 4 cases. In this group, the WCE showed DGTT in 2 cases, diffuse SB mucosal erosions in 2, and focal erosion in jejunum and ileum in 1 patient respectively. In 1 patient in whom the WCE only showed DGTT, the study was repeated 1 week later showing extensive diffuse SB mucosal ulcers. aCT scan was performed in 8 patients, 4 on each group. In the severe GVHD group, 100% showed gut wall thickening.

Discussion: WCE is an excellent method for assessing GI GVHD, especially for those cases with high suspicion of GVHD and negative studies, and a useful technique to confirm extensive involvement of severe cases. Currently we are evaluating use of WCE in assessing response to therapy.

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Expression of toll-like receptors on peripheral blood cells after allogeneic stem cell transplantation: ongoing results of a prospective study

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Emerging trends emphasize the importance of both innate and adaptive immune system in the response against infections, in the pathogenesis of autoimmune diseases and graft-versus-host disease (GVHD). In the cross-talk between innate and adaptive immune system, pattern recognition receptors such as Toll-like receptors (TLRs) play a key role. TLRs recognize common protein, carbohydrate or DNA/RNA pattern motifs leading to signalling for cytokine production and T cell and dendritic cell maturation. These receptors may act as tuner of inflammatory and immunologic reactions. Very little is known about the expression and the function of TLRs in vivo in patients who underwent to allogeneic stem cell transplantation (SCT). The aim of this study was to evaluate the expression of TLRs on lymphocytes and monocytes in relation to infection (CMV and HHV-6, especially) and the onset of GVHD.

The expression of TLRs on T cells and monocytes was analysed by flow cytometry at day +30,+90 after SCT and at the onset of GVHD. The expression of receptors for lipid-based pathogen-associated molecular patterns (PAMPs: TLR 1,2,4 and 6 surface receptors), receptors for nucleic acid based PAMPs (TLR 3,7,8 and 9 located in cytoplasmic compartments), TLR5 and, TLR10 (surface receptors) was evaluated as mean fluorescence intensity (MFI). Since the beginning of the study, we have analyzed data of 14 healthy donors and 22 patients. Median age was 46 years (range, 25-64) and 14 patients were male.

Acute GVHD developed in 12 patients (10 cases with grade ≥2). Patients without acute GVHD after SCT and healthy donors showed different MFI of TLR6 on T cells (2,9±2,3 vs 1,3±1,05 p=0,03). TLR-1 and -3 expression was significantly increased on monocytes in patients with acute GVHD in comparison to those without GVHD (51,4±42,7 vs 16,2±19,6 p=0,02; 8,4±1,02 vs 3,9±2,7 p=0,0001). The levels of TLR5 on T cells were higher in patients with acute GVHD (3,9±2,4 vs 2,2±1,0 p=0,05).

In our study, a different expression profile of TLRs was found in healthy donors, in patients after SCT without acute GVHD and in those with GVHD. These results suggest that the innate immune response via TLRs activation could be involved in the development of GVHD. The assessment of a larger number of patients and the functional analysis of TLRs, still ongoing, would be useful to understand the complex interplay between pathogens, self or non-self DNA and RNA and the immune system after SCT.

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ROR-γ and IL-17A mRNA levels closely correlate in stimulated mononuclear cells in HSCT patients

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CD4 positive lymphocyte subpopulations include more recently described Th17 cells. The hallmarks of these cells is IL-17 positivity and the expression of ROR-γ transcription factor. In our previous study we showed that proportions of IL-17A producing cells in blood elevated early post transplant decrease when aGvHD is clinically apparent. To support the notion that IL-17A producing cells are critical for aGvHD process we investigated IL-17A and ROR-γ mRNA in mononuclear cells purified from blood of patients obtained at the time of hematological recovery (10-20 day post HSCT).

Expression of IL-17A and ROR-γ were analyzed in non stimulated and stimulated with BD Leukocyte Activation Cocktail (containing PMA, Ionomycin and Brefeldin A) mononuclear cells (PBMC) of 39 patients after hematopoietic stem cells transplantation (22 females, 17 males; 16 transplanted from unrelated donors, 23 from siblings). The relative expressions of IL-17A and ROR-γ were measured with the use of Real-Time PCR technique and calculated with respect to 4 references genes (β 2 microglobulin, β actin, ABL and hypoxanthine-guanine phosphoribosyltransferase).

We found:

- higher expression of IL-17A (0.2102±0.1177 vs. 0.0082±0.0056, p<0.0001) and ROR-γ (0.2159±0.0775 vs.0.0606±0.0119, p=0.0001) mRNA in mononuclear cells after stimulation a as compared to fresh cells;
- lower expression of ROR-γ mRNA in non stimulated (0.027±0.0005 vs. 0.038±0.014, p=0.091) and stimulated

(0.007±0.0004 vs. 0.082±0.019, p=0.067) mononuclear blood cells patients at the time of clinically apparent aGvHD as compared to counter-partners lacking aGvHD;
- significant correlation between ROR γ and IL-17A expressions in stimulated cells (r=0.723, p<0.0001).
Conclusions: Response to stimulation results in the increase of both IL-17A and ROR-γ mRNA in PBMNC.
Early aGvHD cases have lower expression of ROR-γ at the time of aGvHD manifestation.

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The clinical and histopathological characteristics of oral GvHD after allogeneic stem cells transplantations with the FLU/MEL conditioning regimen

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Introduction: Oral cavity can be affected with chronic GVHD in 38-46% transplanted patients and in 54-80% patients with ongoing chronic GVHD. Oral acute GVHD is less common. Characteristic of oral GVHD in the FLU/MEL conditioning regimen have not yet been published in details.
Methods and patients: Oral GVHD was prospectively observed in 71 patients after allogeneic SCT with the FLU/MEL (fludarabine 120 mg/m² and melphalan 140 mg/m²) conditioning regimen in 1/2005-12/2007. The median duration of the observation was 13 (0,2–65) months. The patients: median age 56 (23-68), males 51%, HLA identical donor in 57/71 (80%), peripheral stem cells graft in 100%. The intensity of oral GVHD was scored using the National Institutes of Health - NIH criteria: 0=no symptoms, 1=mild symptoms not limiting oral intake, 2=moderate symptoms and partial limitation of oral intake, 3=severe symptoms with major limitation of oral intake.
Results: Oral acute GVHD developed in 5/71 (7%) patients and in 17% of these with systemic acute GVHD, median onset on day +85 (40–125) and median duration of 24 (7–54) days. The intensity of oral symptoms (NIH) was gr.1 in 5/5 (100%). Mild lichenoid changes on buccal mucosa were observed in 3/5 (60%) patients. Oral chronic GVHD developed in 22/62 (33%) patients and in 22/30 (73%) with chronic GVHD. Oral symptoms developed with the median onset on day +237 (107–540), persisted for 188 (11–665) days and resolved on day +420 (178–900) post-transplant. Lichenoid changes were in 22/22 (100%), erythema in 8/22 (36%), defect-pseudomembrane in 12/22 (54%) and atrophy in 3/22 (13%) patients. The intensity of oral symptoms (NIH): gr.1 16/22 (73%), gr.2 4/22 (18%), gr.3 2/22 (9%). The oral GVHD reoccurrence was observed in 7/22 (32%) patients. Tissue samples of bucal mucosa were excised in 3 patients with clinically acute and 9 patients with chronic oral GVHD. The interface lymphocytic inflammation, apoptotic bodies and satellite necrosis were typical findings in all of the samples.
Conclusions: Even though the oral chronic GVHD was mild in the majority of the patients, the mucosal affection can be considered as clinically significant due to its rather prolonged duration, discomfort and pains in some patients. It is also important to be aware of other complications that can mimic the oral GVHD – local toxic or allergic reactions, viral infection, lichen ruber planus and dental-prosthesis lichenoid reaction.

P489

FECAL calprotectin as non-invasive biomarker of acute and chronic GvHD in allogeneic stem cell transplantation

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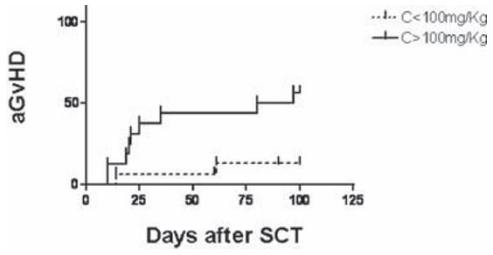
Graft versus Host Disease (GvHD) is one of the major life threatening complication after allogeneic stem cell transplantation (SCT). Recently, the application of proteomic tools has allowed to identify protein pattern of biological samples in patients (pts) with GvHD.
Several authors have investigated the role of fecal calprotectin (a neutrophil cytosolic protein) (FC) in patients affected by inflammatory bowel disease. So, level of fecal calprotectin seems to have a proportional correlation to the degree of immune inflammation of the intestinal mucosa.
The aim of our study has been to evaluate the role of FC level as a possible non-invasive marker of GvHD.
Since February 2009 to July 2010, we enrolled 39 pts, M/F 20/19, with a median age of 49 years (range 17-65), submitted to an allogeneic SCT in our centre. Underlying diseases were: CLL (2 pts), ALL (5 pts), MDS (6 pts), NHL (5 pts), AML(18 pts), HD (1pt), IMF (1pt) and plasma cell leukemia (1 pt). Twelve pts received myeloablative conditioning and 27 received reduced intensity conditioning. Graft was obtained from sibling donor and MUD in 22 and 17 pts respectively. Stem cell source was PB in 37 pts, CB in one pt and BM in another one. GvHD prophylaxis was performed with cyclosporine A (CSA) in 3 pts, CSA+MTX in 18 pts, CSA+MMF acid in 18 pts; six pts received also ATG and 3 pts Campath-1H.
ELISA test for FC level was performed on day +30, +60 and +90 after SCT and at the onset of GVHD.
Pts received a median number of CD34+ $7.10x10^6$/Kg (range 1.47-17.4). Median time to neutrophil engraftment (PMN>$0.5x10^9$/L) was 15 days (range 9-34), while a spontaneous platelet recovery (PLT>$20x10^9$/L) was achieved after a median time of 13 days (range 2-46).
TRM at 100 days was 23%: 2 pts died for aGVHD, 2 pts for sepsis, 1 pt for pulmonary aspergillosis and 4 for progression disease.
Seventeen pts developed aGVHD (43%) (5 pts grade I-II and 12 pts grade III-IV) and 12 pts developed cGVHD (4 pts limited and 11 pts extensive).
FC median level in the first 100 days after SCT was significantly higher in the group with aGVHD (median value 218 mg/kg vs 53 mg/kg, Fisher exact's test p=0.0002, Fig 1) and in the group of pts with cGVHD (median value 187.5 mg/kg vs 59 mg/kg, Fisher exact's test p= 0.0472). FC was also higher in extensive than in limited cGvHD (203 mg/Kg vs 23 mg/Kg, Fisher exact's test p=0.0410).
Despite the small number of pts, we propose the FC level as a marker of GVHD activity with a predictive role on cGVHD onset.



P490

Assessment of lymphocyte infiltrates in liver, intestinal and skin biopsies in patients with GvHD

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Some authors have previously reported correlations of CD4, CD8 and CD25 positive cells intestinal infiltration with the severity of GVHD, the use of corticosteroids or certain genotypes. Few authors have assessed the pattern of lymphoid infiltration in skin GVHD. We therefore performed a retrospective analysis of CD20, CD8, CD3, CD25, FoxP3 positive cells and

Granzyme B expression in liver, intestinal and skin biopsy from 15 patients with clinical acute GVHD. Some intestinal biopsies were unrated because of too small size of the fragments collected.
In skin biopsies examinated, mostly CD3 positive T lymphocytes regulator type (Fox P3 positive) were found near the apoptotic keratinocytes (satelitosis), at the dermal-epidermal limit and perivascular in the superficial dermis. Some of the T cells located at the dermal-epidermal limit were larger activated CD25 positive cells. No CD20 positive B cells were found. These aspects were correlated with a clinical severe GVHD. In the biopsies examinated from the intestine and liver rare cells were FoxP3 positive, disposed near the intestinal epithelium or portal, near the billiary ducts. The CD25 positive cells were rarely found and isolated. Granzyme B expression was not found. Our data indicate that while in clinical severe skin GVHD regulatory T cells predominate in the lymphoid skin infiltrate, even in clinical severe digestive GVHD regulatory T cells priming occur rather in the lymph nodes than in the GVH-tissue. This study was conducted in the frame of the National Research Project PNII-42172/2008.

P491

Cyclosporin monitoring at 2 hours from oral intake leads to less toxicity and better efficacy in patients undergoing haematopoietic stem cell transplantation and immunosuppressive treatment for severe aplastic anaemia

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Calcineurin inhibitors are widely used as immunosuppressive drugs, and there is still a discussion about the optimal blood levels of Cyclosporin A (CsA), balancing safety and efficacy. Most of the commonly used pharmacokinetic measurements cannot predict the individual biological effects of the immunosuppressive drug. Data obtained among Solid Organ Transplant settings, demonstrated a higher reliability of drug serum level after 2 hours from oral intake (C2) as regards to absorption and needed oral doses in the specific patient.
Aim: To estimate the safety and efficacy of tihs strategy, we investigated the pharmacokinetics of CsA delivered by twice-daily oral administration to assess if CsA blood level at 2 h from oral intake is a more reliable tool than the pre-intake level to prevent or reduce toxicity (neurotoxicity, nephrotoxicity, TTP, capillary lake syndrome, hypertension, hirsutism) in Hematopoietic Stem Cells Transplantation (HSCT) and Severe Aplastic Anemia (SAA) patients, and to tailor the required dose in each patient.
Patients and methods: From February 2008 to December 2010 we retrospectively enrolled 56 patients (51 post HSCT and 5 treated for SAA). Serum level of CsA at time 0 and after 2 hours from oral intake (1074 samples), creatinine serum levels, drug toxicity, graft versus host disease (GvHD) and CMV reactivations were collected. Oral dose of CsA was modified according to C2.
The results were compared with an hystorical control group of 20 patients (18 after HSCT and 2 cases of SAA) treated from January 2006 to December 2007. In this group the oral dose was adjusted according to pre-intake CsA serum level.
Results: The whole incidence of side effects was lower in the study group (45% vs 50% control group). The mean oral dose administered in study group was considerably lower than hystorical group 2,32 mg/kg (range 0,83-8,19) vs 5,37 mg/Kg (range 2,51-10,4) whereas the same incidence of acute GvHD (grade I-II) and limited chronic GvHD was found in HCT patients of both groups. Interestingly fewer cases of CMV reactivations were found in our study group (23% vs 40%).
Conclusions: These data suggest that dose adjustment of CsA according C2 allows to treat patients with lower dose obtaining a same control of GVHD, and this modality could reduce toxicity risk.
The number of patients doesn't allow to draw final conclusions, prompting the need to further prospective studies.

P492

Time interval from chronic graft-versus-host disease onset and monocyte peripheral blood count count are important predictive factors for response in patients with steroid resistant graft-versus-host disease undergoing extracorporeal photopheresis

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Chronic graft vs host disease (cGVHD) affects 50% of successfully engrafted patients and has a negative impact on the morbidity and quality of life, as well as in nonrelapse mortality. Corticosteroids with or without calcineurin inhibitors are the standard of care for initial treatment of cGVHD but a substantial number of patients do not respond to them. A variety of immunosuppressive (IS) treatments have been tested in steroid resistant cGVHD, among them extracorporeal photopheresis (ECP). We evaluated the efficacy and safety of ECP in a series of 45 patients (pts) with relapsed or refractory cGVHD. ECP was started at a median of 275 days (32-3851 days) after the onset of cGVHD. The Therakos XTS system was utilized and pts were treated on 2 consecutive days (one cycle) weekly for 3 months, followed by an individual progressive tapering schedule according to the course of cGVHD or until maximum response was obtained. All pts had at least a 12-months period of follow-up (FU) after ECP was started.
Three months after the start of ECP, 2 pts had a complete response (CR) and 24 a partial response (PR). At 6 months, 5 and 27 pts had CR and PR respectively. At 9 months, 8 and 23 pts were in CR and PR respectively. At 1 year, 18 had a CR, including 7 who were free from systemic IS treatment, 20 had a PR and only 1 had no response. At last FU 29 and 15 pts were respectively in CR and PR and 26 were free from IS treatment. Time from cGVHD onset less than 121 days was significantly associated with a higher response rate (OR = 8, 95% confidence interval 1.75-36.4, P = 0.003) and a higher peripheral blood monocyte (PBMC) count (>0.4x10^9/L) was associated with a lower response rate (OR = 0.25, 95% confidence interval 0.07-0.088, P = 0.027). During the time to treatment evaluation no patient died as a result of procedure-related adverse events. After this time global mortality was 22.2%, with cGVHD-related mortality being 6.6%. Estimated overall survival at 5 years is 64% (Fig. 1).
Of note, pts achieving a CR had a significantly lower number of PBMC at the start of treatment. Monocyte derived dendritic cells induce T-helper cells to differentiate into Th1 cells. Whether this

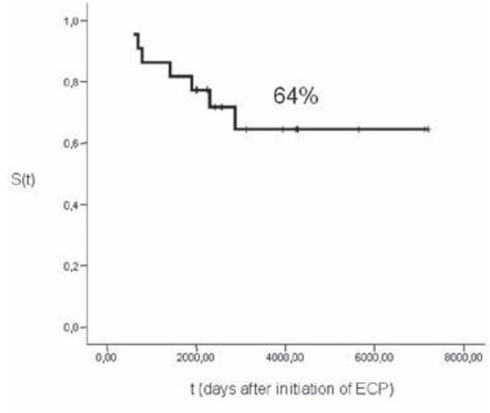

Figure 1. Overall survival at 5 years

may affect the immune response to treatment remains speculative but might explain this observation.
Our results suggest that the effects of ECP may be cumulative and delayed in time, with pts responding or continuing to improve as long as 12 months after the start of treatment.

P493

Pre-transplant rituximab is associated with reduced rate of acute GvHD after RIC-AlloSCT in lymphoma patients

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Both B and T cells are implicated in the pathophysiology of graft-versus-host disease (GvHD) after allogeneic hematopoietic stem cell transplantation (AlloSCT). Anti-thymocyte globulin (ATG) administered during conditioning has been shown to reduce both acute and chronic GvHD, while rituximab has been suggested to reduce incidence of GvHD: previous studies indicated a reduced incidence of acute and/or chronic GvHD, but sometimes with controversial results. Here we present results from a retrospective, monocenter analysis on a selected population of lymphoma patients undergoing reduced-intensity (RIC) AlloSCT, with or without ATG in the conditioning regimen.
Adult patients receiving RIC-AlloSCT from a HLA-identical sibling donor for relapsed CD20+ lymphoproliferative disease at our Institution were included in the analysis. Rituximab had been administered in association or not with chemotherapy, according to each patient's treatment strategy.
57 patients transplanted between Avril 1999 and November 2009 were included in the study. Thirty-nine patients received ATG and 18 did not. Of these 57 patients, 32 were treated with rituximab, ending at a median (range) of 43 (5-177) days prior to transplant. Median follow-up for all patients was 749 days (146-4051). No significant differences in pre-transplant variables existed between patients receiving rituximab (RTX group) vs. those without rituximab (no-RTX group), with the exception of more FL in RTX group and more CLL in no-RTX group (p=0.02). In the cohort of patients receiving ATG, rituximab used at a dose ≥ 375 mg/m^2 (n=10) within three months prior to AlloSCT was associated with a reduced rate of grade 3-4 aGvHD compared with no rituximab (n=29): 0/10 vs. 7/29 (p=0.08). Reduction of grade 2-4 aGvHD rate was also observed: 1/10 vs. 14/29 (p=0.03, figure 1). Among the 18 non-ATG patients, we did not observe any protective effect on aGvHD by RTX group. Similarly, no effect on cGvHD appeared in the two cohorts.
Present data suggest a role of rituximab administered before AlloSCT in reducing the incidence of aGvHD. This effect was more pronounced when administration of ATG was performed during conditioning regimen, inviting considering a possible synergistic effect of both T- and B-cell depletion in preventing GvHD. Although retrospective and small-sized, the present analysis, conducted on a quite homogeneous population of patients, invites to further investigations on larger number of patients.

P494

Pravastatin after allogeneic haematopoietic stem cell transplantation: better tolerated than expected but without significant influence on chronic graft-versus-host disease

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Objectives: As an approved drug for therapy of hyperlipidemia in patients (pts) taking immunosuppressive therapy after organ transplantation pravastatin is used rarely after allogeneic hematopoietic stem cell transplantation (HSCT), because of expecting adverse effects above average. One reason for this anticipation is the extensive comedication. Another reason are therapy related problems as myopathy or elevated liver enzymes, which are expected to worsen. Because of the recurring question of influence of statins on graft versus- host- disease (GvHD) pravastatin was also evaluated on this focus.
Patients and methods: 50 consecutive pts after myeloablative HSCT (25 female, 25 male, median age 53 years) with a median pravastatin intake of 257 days were analyzed retrospectively and compared to a matched pair group of 50 HSCT pts without pravastatin medication. Pts with acute and chronic myeloid and lymphatic disorders and myelodysplastic syndroms were included, 17 pts had related, 33 pts unrelated donors. 6 pts showed no, 18 limited and 26 extended cGvHD. Drug tolerance was documented by continuous monitoring of the clinical status, measurement of creatine kinase and liver enzymes. GvHD was clinically evaluated, dosage of immunosuppressive drugs (cyclosporine, prednisone) as well as blood eosinophilia was recorded. Relapse and survival was monitored.
Results: In 4 cases with absent or limited cGVHD pravastatin was discontinued. 3 pts showed muscular pain without change of creatine kinase, 1 had no clinical symptoms but developed increased creatine kinase and liver enzymes (ctc grade 3 and 2). During time of taking pravastatin 30 (vs. 33 in control group) of 50 pts showed no change of cGvHD, 17 (vs. 10) had an improvement and 3 (vs. 7) an impairment. 13 pts reduced the dosage of cyclosporine, 34 did not change and 3 increased it. Regarding prednisone 14 pts reduced the dosage, 29 did not change and 7 increased it. Results did not significantly differ from the control group. Out of 6 pts with eosinophilia 5 reached normal results, 1 increased. During statin medication 5 pts relapsed, 2 died – one because of relapse, one by infection. In the control group 5 pts relapsed, 4 died – 2 because of relapse, 2 by GvHD.
Conclusions: Pravastatin is a well-tolerated drug after HSCT revealing comparable side effects as in pts without HSCT. There seems to be no significant influence on the course of cGvHD although effects may be seen only after long-term intake.

P495

Tear film osmolarity in chronic ocular GvHD patients

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Purpose: to evaluate tear film osmolarity in patients with chronic GVHD.
Methods: twenty-nine consecutive patients who underwent allogenic hematopoietic stem cell transplantation(HSCT)received complete ophthalmologic examination including: visual acuity, tonometry, Schirmer test, Break-up time (BUT), green lissamine conjuctival and corneal staining test (Oxford scale), and ocular surface disease index score evaluation(OSDI). All patients underwent tear film osmolarity measurement with the TearLab Osmolarity System instrument.
Results: There were 14 male patients (48.3%) and 15 female patients (51.7%) with a mean age of 45 years (39-51). 83% presented a dry eye disease (DED) characterize by: OSDI mean 43.5 (33.3-53.6), mean Schirmer test 5.9 mm (3.3-8.4), mean BUT of 5.9 sec. (3.3-8.4). Mean osmolarity value was 316 mOsm/L (290-370).
Conclusions: Tear osmolarity cut off values is 316±1 mOsm/L; higher osmolarity values can correlate with dry eye disease severity. In our sample tear osmolarity showed correlation with Schirmer Test, tear film BUT and lissamine green corneal staining. No correlation were found with visual acuity, OSDI and conjunctival and corneal staining.
Our results showed a correlation between higher values of Osmolarity and DED in ocular cronic GVHD. Assessment of tear film osmolarity may provide an objective, rapid and reliable test for determining dry eye disease in patients with ocular chronic GVHD.

P496

CicloMulsion®, a novel Cremophor® EL-free ready-to-use formulation of cyclosporine for intravenous use, is bioequivalent to Sandimmune® Injection and exhibits less adverse effects – Final study report

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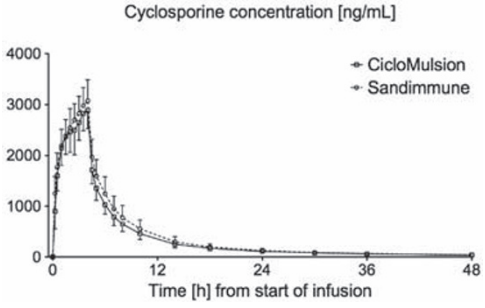
Introduction: Cyclosporine (CsA) is widely used to prevent rejection of grafts after transplantation, to treat graft-versus-host disease and as management of a number of autoimmune diseases. The intravenous (IV) form currently on the market uses Cremophor EL (CrEL) as carrier medium. CrEL is known to cause hypersensitivity reactions in some patients, ranging from skin reactions to dyspnea, anaphylactic shock and death. The objectives of this study were to assess the pharmacokinetics of a new CrEL-free formulation of CsA, CicloMulsion, compared to a marketed product Sandimmune Injection and to compare the profiles of adverse effects between the two formulations.
Materials and methods: In this open-label, laboratory-blind, subject-blind, randomized, single-dose, two-period crossover study, 52 Caucasian and Non-Caucasian male and female volunteers were treated with 5 mg/kg of each of the two formulations of CsA as an infusion over 4 hours. Serial blood samples were obtained and analyzed for CsA-concentration. Pharmacokinetic and bioequivalence comparisons according to current US and European guidelines were performed with calculations of point estimates and 90% confidence intervals for the test/reference geometric least square mean ratios of relevant variables.
Subjects were monitored for adverse events with blood sample screenings, vital parameters, physical examinations and by questioning.
Results: The geometric mean ratios for CicloMulsion/Sandimmune (90% confidence interval) of the primary variables were 0.899 (0.877-0.922) for Area Under Curve (0 to infinity) and 0.948 (0.924-0.972) for Maximum blood CsA concentration. All additional variables analyzed also fell well within the bioequivalence range of 0.80-1.25.
One anaphylactoid and one anaphylactic reaction, both classified as serious adverse events (SAE) were reported, after treatment with Sandimmune. No SAEs were recorded with CicloMulsion. The proportion of overall adverse events was significantly higher when Sandimmune was used.
Conclusion: CicloMulsion, a novel CrEL-free formulation of CsA, is bioequivalent to Sandimmune Injection and exhibits less adverse events. The use of CicloMulsion should thus be considered when treating patients with IV CsA in order to avoid hypersensitivity and anaphylactic reactions.



P497

Extracorporeal photopheresis with the new Cellex system in paediatric and severely ill patients

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We have treated since October 2009 26 patients (8-73 years of age) with the new CELLEX system for ECP (2-22 treatment sessions per patient). 9 patients were below 18 years of age.Body weight was 16.2-84 kg (with 7 patients below 40 kg). Indications for treatment were severe acute or chronic graft-versus-host disease (GVHD) in 17 patients, cutaneous T cell lymphoma in 3 patients and refractory acute rejection after solid organ transplantation or severe autoimmune disease in 6 patients.
During the year 2010 several technical changes have improved the CELLEX system. In our experience it is an effective and safe treatment modality. Main advantages of the CELLEX system are a markedly reduced extracorporeal blood volume, especially if the double needle modality can be used. Treatment time is reduced substantially (up to half) compared to the conventional UVAR XTS system for ECP. Furthermore conventional Port systems and Hickman catheters already in place can be used as venous access. In adult patients often times a peripheral 16 gauge needle is sufficient to achieve good blood flow.
Of the 17 patients with GVHD 11 suffered from Grade 3-4 acute graft-versus-host disease (revised Glucksberg score). With an intensified ECP schedule of 2 treatments weekly 9 patients improved with their skin, liver and intestinal disease. Their corticosteroid dose could be reduced or discontinued.
6 patients suffered from chronic GVHD or overlap syndrome with involvement of lungs, muscles, skin, eyes and gut. Their disease severity could be categorized as “severe” according to usual clinical scores. With an intensified ECP schedule in the beginning and long-term continuation of treatments every 2 weeks a variable degree of improvement in the disease manifestations could be accomplished. Corticosteroid dosage could be reduced or discontinued.
In summary the new CELLEX system for ECP showed treatment results as good as the conventional UVAR XTS system in our patients. With low extracorporeal blood volume, shortened treatment time and easy to accomplish venous access the online ECP treatment for paediatric and severely ill patients has become feasible.

P498

Risk factors for acute and chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation in paediatric patients

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Introduction: Despite progress in strategies for successful allogeneic hematopoietic stem cell transplantation (allo-HSCT), graft-versus-host-disease (GVHD) remains the most significant cause of non-relapse morbidity and mortality. In this study, we analyzed factors associated with acute or chronic GVHD after allo-HSCT in a single center.
Patients and methods: A total of 142 patients (79 males and 63 females) with a median age of 9.3 years (range 0.9-19.2) who underwent allo-HSCT between October 2004 and December 2009 were evaluated. The source of stem cells was bone marrow (BM) in 53, peripheral blood (PB) in 38 and cord blood (CB) in 51 cases.
Results: The cumulative incidence (CI) of acute GVHD was 0.42 for grade II-IV and 0.21 for grade III-IV. Male recipient (P=0.005), female donor/ male recipient match (P=0.024) and higher donor age (≥ 30 years) (P=0.001) were risk factors for grade II-IV acute GVHD on univariate analysis. Male recipient (P=0.020) remained significant on multivariate analysis. Male recipient (P=0.045) and higher donor age (≥ 30 years) (P=0.001) were risk factors for grade III-IV acute GVHD on univariate analysis.

Higher donor age (≥ 30 years) (P=0.023) remained significant on multivariate analysis. In unrelated transplants, tacrolimus-based GVHD prophylaxis instead of cyclosporine lowered the CI of acute GVHD (0.57 vs 0.35) to the comparable level with that in related GVHD. The CI of chronic GVHD was 0.29. Previous acute GVHD (P<0.001) and eosinophilia (P=0.006) were risk factors for chronic GVHD. Use of total body irradiation (TBI) (P=0.004) and absence of anti-thymocyte globulin (ATG) (P=0.009) in the conditioning regimen were also associated with the risk of developing chronic GVHD. However, the effect of each factor was not clear due to the fact that most patients had either TBI or ATG.
Conclusion: Our analysis showed several risk factors for acute and chronic GVHD. These data could be useful in predicting the risk of GVHD.

P499

To perform or not to perform T-cell depletion in unrelated stem cell transplantation?

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Aim: To compare two groups of patiens undergoing unrelated allogeneic stem cell transplantation with or without in vivo T–cell depletion with pretransplant administration of Thymogloubuline (Genzyme).
Methods: We retrospectively evaluated two groups of consecutive patiens, who underwent stem cell transplantation at our institution. From year 2005 we abandoned T cell depletion in unrelated stem cell transplantation according to current institutional guidelines. After analysis of the results we decided to administer in vivo T-cell depletion with Thymoglobuline (Genzyme) in dose 2,5 mg/kg at days -3 to -1 (from 2007). The patients were included only if they had at least 1 year follow up.
Results: There were 32 patients in the group without T-cell depletion and 44 patients in T-cell depleted arm. There was a significant difference in overall mortality and mortality due to refractory GVHD in favor of T cell depletion. Overall mortality was 72% and 43% (p=0,01) and mortality due to refractory GVHD was 22% and 0% (p=0,0011) respectively for the group without and with T-cell depletion. There was no significant difference in the incidence of GVHD (all grades), in the incidence of relapse and in the mortality caused by relapse of the primary disease. Incidence of GVHD, incidence of relapse and mortality due to relapse was 69% and 64%, 41% and 32%, 25% and 20% respectively in non T-cell depleted and T-cell depleted arm. We did not observe increase in severe infections with T-cell depletion. There was clear benefit in the quality of life in T-cell depleted arm.
Conclusion: We conclude, that there is a clear benefit of in vivo T-cell depletion in patients undergoing unrelated stem cell transplantation and that there is unjustified fear of larger amount of relapse and infection.

P500

Extracorporeal photochemotherapy in the treatment of acute and chronic GvHD: experience from Rome Transplant Network

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Background: Graft-versus-host disease (GVHD) is a major complication of allogeneic stem cell transplantation and remains a main cause of non relapse mortality. Extracorporeal photochemotherapy (ECP) is an immunomodulatory therapy showed to be effective in the treatment of GVHD. We present our results in the use of ECP as treatment of acute or chronic GVHD in patients referred for transplant to RTN.
Patient and methods: 36 patients (25 M/ 11F; median age 36 years, range 6 to 62 years) with acute (n=10) or chronic (n=26) GVHD were included in the study. For ECP an off-line system was used. Briefly, mononuclear cell (MNC) concentrate was collected through a single leukapheresis; cell concentrate was then UVA irradiated after injection of 8-MOP. Two different protocols were used: patients with A-GVHD underwent ECP three times weekly over 2 weeks, twice weekly until week 10, twice every two weeks until week 26 and twice monthly during the following 4-8 months; patients with C-GVHD underwent ECP twice weekly until week 8, twice every two weeks until week 24, twice monthly during the following 4-8 months.
Results: A total of 1132 ECP procedures have been performed. The median number of ECP courses was 26 (range 12-56) for A-GVHD and 32 (5-55) for C-GVHD. The median number of nucleated cells treated was 5.4 x 10^9 (range 0,18 – 28,2). Of 36 patients, 27 (75%) are surviving and 9 died (4 of 10 with A-GVHD and 5 of 26 with C-GVHD. The causes of death were: 3 liver GVHD, 2 lung GVHD, 1 leukemia relapse, 3 pneumonia). Of 27 surviving patients, 15 (55%) (1 A-GVHD and 14 C-GVHD) achieved a complete response and 9 (33%) (2 A-GVHD, 7 C-GVHD) had a partial response. ECP treatment was discontinued in 3 cases for leukemia relapse. Immunosuppressive therapy combined with ECP was progressively and rapidly discontinued in 11 patients. Only 12 out of 1132 (1%) procedures were interrupted: for nausea and vomiting or for complications related to vascular access.
Conclusion: Our data confirm the efficacy and safety of ECP, particularly in the treatment of C-GVHD (response in 21 out of 26 cases), may represent an important alternative in case of steroid intolerance and contribute to accelerate discontinuation of the immunosuppressive therapy.

P501

Graft-versus-host disease prophylaxis with high-dose cyclophosphamide after myeloablative peripheral blood stem cell transplantation for acute myeloid leukaemia

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Introduction: Graft versus host disease (GVHD) is a major complication of allogeneic hematopoietic stem cell transplantation. The optimal approach for GVHD prophylaxis remains uncertain. Acute GVHD still occurs in 35-55% with standard regimens. Moreover, they are not as effective in reducing the incidence of chronic GVHD and may impair immune reconstitution. High dose cyclophosphamide is a promising novel strategy. Here, we report our own experience with this regimen in comparison to prophylaxis with methotrexate in acute myeloid leukemia patients.
Methods: All patients received peripheral blood stem cells from HLA matched related donors after myeloablative busulphan and cyclophosphamide conditioning. High dose cyclophosphamide (50 mg/kg/day) was given on days +3 and +4 to a group of 13 patients. Methotrexate 15 mg/m^2 on day +1, 10 mg/m^2 on days +3, +6 and +11 was given to the other group (n=13). Both groups also received cyclosporine 6,25 mg/kg/12 hours iv beginning from day -1 and the dose is adjusted according to blood levels. Results: The median age was 41 years (range 28-55), total CD34+ cell count was 5.2 (range 2.1-12) x10^6/kg, time to neutrophil engraftment was 18 days (range 14-36), time to platelet engraftment was 15 days (range 9-34) in cyclophosphamide group. The median age was 41 years (range 22-58), total CD34+ cell count was 3.9 (range 2.3-8.4) x10^6/kg, time to neutrophil engraftment was 18 days (range 10-27), time to platelet engraftment was 14 days (range 10-50) in methotrexate group. All patients were in complete remission except 1 patient in cyclophosphamide and 2 patients in methotrexate group. Acute GVHD was observed in 7 of 13 patients

and maximum grade was 2 in cyclophosphamide group. Acute GVHD was observed in 9 of 13 patients and maximum grade was 3 in methotrexate group. Chronic GVHD was observed in 4 patients in cyclophosphamide and 7 patients in methotrexate group. Hemorrhagic cystitis was observed in 3 patients in cyclophosphamide and 1 patient in methotrexate group. Need for antibacterial and antifungal therapy was similar between 2 groups.
Conclusion: High dose cyclophosphamide can be used for GVHD prophylaxis with less severe GVHD but a slightly higher hemorrhagic cystitis incidence.

P502
Pre-transplant low-dose ATG is associated with reduced transplant-related mortality and improved clinical outcome in patients receiving allogeneic HSCT from unrelated and partially matched related donors
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We investigated the outcome of patients with hematological malignancies who received an allogeneic stem cell transplantation (SCT) from alternative donors after a conditioning including low dose rabbit antithymocyte globulin (ATG) (Thymoglobulin). Ninety adults received ATG 7.0-7.5 mg/Kg over 2 days (–4, –3) with myeloablative (n=55) or reduced intensity conditioning (RIC) (n=35) and underwent an allogeneic SCT from an unrelated donor (n= 82) or a partially matched related donor (n=8). In addition to ATG, 84 patients (94%) received cyclosporine and methotrexate for GVHD (graft versus host disease) prophylaxis. The source of stem cell was the peripheral blood in 73% of the cases. One patient died before engraftment and 4 experienced secondary graft failure. Median time to neutrophil engraftment was 17 days (range 11-48 days); at day +30, 93% of the patients showed full donor chimerism. The cumulative incidence of acute GVHD grade II-IV was 23% (95% CI14%-32%) and did not differ between patients receiving conventional and RIC (p=0.32); similarly, the 1-year cumulative incidence of cGVHD was 49% (95% CI 38%-60%) and was not different between the two groups (p=0.11). Blood stream infections occurred in 16 patients (18%), and gram-positive organisms accounted for 62% of bacteremias. An invasive fungal infection occurred in 9 patients (10%) and CMV reactivation developed in 55% of the subjects. The 3-year cumulative incidence of relapse was 34% and did not differ between conventional and RIC HSCT recipients (p=.61). The 100-day and the 1-year cumulative incidence of TRM were 8% (95% CI, 2%-13%) and 15% (95% CI, 8%-23%) respectively.Six patients (11%) died of transplant-related complications after myeloablative compared to 8 (23%) after RIC transplantation, although the difference did not reach the statistical significance (p=.11). With a median follow-up of 32 months (range 1-76 months), the 3-year estimated OS was 53% (95% CI, 43%-53%) and was not statistically different between conventional myeloablative and RIC (p=0.38). The 3-year estimated PFS was 38% (95% CI, 22%-54%) and was significantly higher for patients transplanted with conventional regimens (47%; 95% CI, 33%-61%) compared to RIC (38%; 95% CI, 22%-54%) (p=0.02).This study suggests that the incorporation of low doses of ATG into the preparative regimens of patients receiving SCT from alternative donors reduces the risk of acute and chronic GVHD and potentially may improve the final outcome of the patients.

P503
A single-centre experience of the efficacy of extracorporeal photopheresis in the treatment of steroid-refractory acute graft-versus-host disease
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We have used ECP to treat 19 consecutive patients with steroid-refractory acute GVHD post BMT or DLI therapy. Patients were treated with weekly cycles on the Therakos UVAR XTS instrument for a planned minimum of 8 weeks, and continued until maximum response or CR was seen. ECP was then discontinued without tapering. CR was defined as resolution of features of acute GvHD with a reduction of prednisolone dose to 20 mg/day or less.
The grade of GVHD prior to starting ECP was grade II in 4, grade III in 5 and grade IV in 10 patients. 14 patients had skin involvement, 10 liver involvement and 14 gut involvement. 14/19 of patients had 2 or 3 organ involvement. Patients had received a median of 15 days of prednisolone or methylprednisolone at a dose of 1-2 mg/kg prior to starting ECP. The median number of cycles of ECP administered was 8 (2-15). 13/19 (68%) patients completed ≥8 weeks of treatment. 11/13 patients who completed 8 weeks of ECP have responded. Of these, CR was achieved in 9 and PR in 2, one of whom went on to achieve CR with no additional treatment. Response was achieved in 4/4 patients with grade II GVHD, 2/5 grade III GVHD and in 5/10 grade IV GVHD. Response was achieved in 3/5 patients with single organ involvement and in 8/14 with 2 or 3 organ involvement. 9/11 responding patients developed chronic GVHD but in only one case was this extensive. 2 patients went on to receive further ECP for chronic GVHD. 8 patients died of progressive GvHD, 6 of these prior to completing 8 weeks of ECP therapy. The non-responding patients received a median of 3 cycles of ECP (range 2-15). 5/8 non-responders had grade IV GVHD, the rest had grade III. 5/8 patients had stage III or IV gut involvement and 4 had stage III or IV liver involvement. Only 1 had stage IV skin involvement and this patient also had gut and liver GVHD. The predicted survival at 1 year was 52% for the whole group of 19 patients and 84% for the 13 patients who completed 8 weeks of ECP.
In conclusion we have found ECP to be an effective second line treatment for acute GVHD, even for patients with grade IV GVHD who had a 50% response rate. These findings are in agreement with those of Greinix et al, although the majority of responders in our series developed chronic GVHD requiring further immunosuppressive therapy. Our series is also important in that it demonstrated a response to ECP in 4/6 patients who developed acute GVHD post DLI.

P504
Correlation of HSPA1Lexpression with the development of graft-versus-host disease
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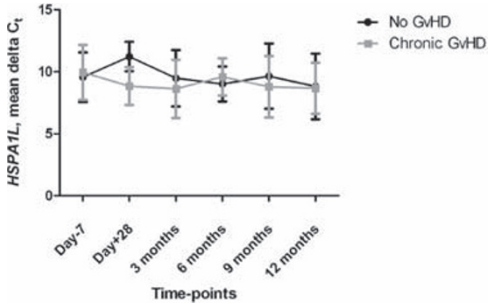
Background: The conserved and well characterized heat shock protein 70s (HSPAs) are expressed by three genes (HSPA1A, HSPA1B, HSPA1L). HSPA1A and HSPA1B are inducible HSPAs (HSP0i) and differ by two amino acids resulting in interchangeable proteins. HSPAs have also been shown to be associated with responses to inflammation and allograft rejection. HSPA1B (HSP0i) had been previously shown to be correlated to severity of Graft-versus-Host Reactivity (GvHR) in a skin explant model by Jarvis et al., 2003. In this study, we have investigated the role of HSPA1L in GvHR and clinical Graft-versus-Host Disease (GvHD) samples to determine whether HSPA1L is involved in the pathogenesis of GvHD. Respectively, the expression

of HSPA1B was also assessed in whole blood to determine whether it is predictive of the severity of GvHD as it is in skin.
Methods: mRNA expression (delta Ct) of HSPA1L was investigated by Taqman qRT-PCR, using skin biopsies from; in vitro skin explant assays at three time points (Days 1, 2 and 3), clinical acute GvHD skin biopsies from allo-HSCT patients and healthy volunteers. HSPA1B (delta Ct) was used as a positive control. Similarly, mRNA expression of HSPA1L and HSPA1B were examined in whole blood samples collected in PAXgene tubes and obtained from allo-HSCT patients at specific time points pre- and post-transplant (Day -7, Day +28, 3 months, 6 months, 9 months and 12 months).
Results: The mRNA expression of HSPA1L was not statistically significant in determining the severity of GvHR or acute GvHD in skin. However, at the time-point Day+28, expression of HSPA1L in whole blood, was shown to be significant in predicting chronic GvHD (p= 0.0117, 95% CI 0.607-4.15) (Figure 1).
Conclusion: The mRNA expression of HSPA1L in skin does not change with development of GvHR or GvHD. However, the expression of HSPA1L in whole blood may be an indicator of chronic GvHD. The expression of HSPA1B in whole blood was not predictive of GvHD unlike its expression in skin as shown previously by Jarvis et al., 2003.



P505
Various therapy schemes of chronic graft-versus-host disease
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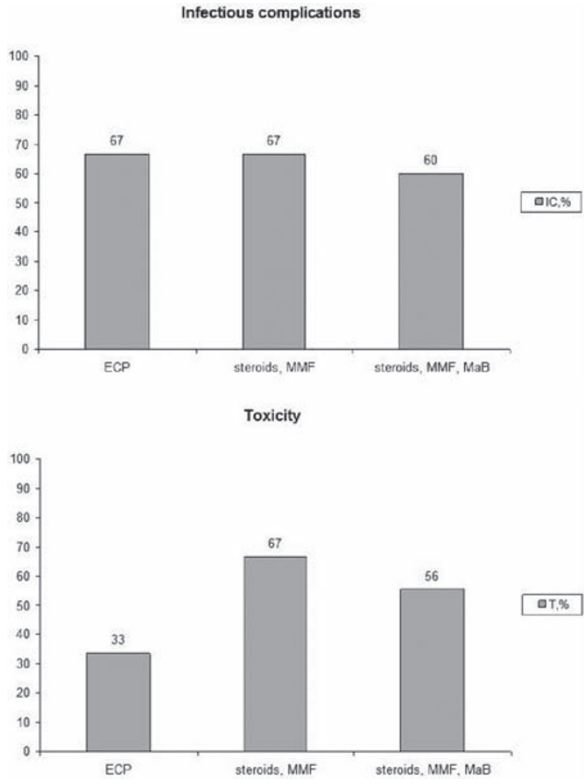
Chronic graft-versus-host disease (cGVHD) is the most serious and common long-term complication of HSCT, occurring in 20% to 70% of people surviving more than 100 days and it represents the major cause of nonrelapse mortality and morbidity in long-term survivors. The optimal treatment scheme for cGVHD combining symptoms control with acceptable rate of toxic and infectious complictions which are a major cause of death in these patients is still under investigation.
The aim: To compare efficiency and complications of various therapy schemes of cGVHD in children and adolescents after allo-HSCT.
Patients and results: 140 children and adolescents, age from 1 to 21 y.o. (median 12 y.o.), which survive more than 100 days after allogenic HSCT (allo-HSCT). All pts received allo-HSCT. Source of HSC were: bone marrow (BM) – 43% (60 pts), peripheral blood (PB) – 43% (60 pts), BM+PB – 13% (18 pts), cord blood – 1% (2 pts). Common frequency of cGVHD was – 46% (65 pts). In group, which use BM, frequency of cGVHD – 41%, PB – 63%, BM+PB – 44%, CB – 0%. Therapy of cGVHD was: 6% (4 pts) received topical steroids, 10% (6 pts) – steroids with MMF, 15% (10 pts) – steroids with monoclonal antibodies, 15% (10 pts) – steroids with MMF and monoclonal antibodies, 15% (10 pts) – steroids with CsA or Tx, 19% (12 pts) – secondary therapy included ECP, 20% (13 pts) – only steroids. We compared effectiveness, infectious complication and toxicity different in different therapy groups (Fig. 1, 2).
Conclusions: The level of infectious complications is identical in all therapy groups. The therapy scheme of cGVHD with ECP is more effective for achieving partial resolution. Complete resolution can be achieved using combination of ECP, immunosuppressive drugs and MaBs. Toxic complications were observed less often in ECP-containing therapy scheme.



P506
Risk factors for severe aGvHD in sibling transplants after reduced-intensity conditioning
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Background: Despite optimal HLA matching in sibling transplantation (SCT), severe acute GVHD (grades II-IV) can occur in about one third of the patients. With the introduction of reduced intensity conditioning (RIC) regimens, less tissue damage and therefore less acute GVHD is expected. In this retrospective study, we wanted to identify factors that were correlated with severe aGVHD after sibling SCT and RIC.
Methods: This study included 150 patients with different diagnoses. Median age was 53 (0.5-77) years. Stem cell source was PBSC in 85% of the transplants and BM in 15%. As GVHD prophylaxis a combination of CsA and MTX was used in most cases (71%). All RIC regimens included Fludarabine which was combined with Busulfan in 39%, TBI 2Gy in 19%, TBI 2x3 Gy +Cy in 12%, Cy in 23% and Treosulfan in 7% of the transplants. Anti-thymocyte globulin (ATG) was given to 45% of the patients.
Results: The incidence of aGVHD II-IV was 32%. In multivariate analysis, the only two factors that correlated with aGVHD II-IV were patient age (HR 1.51, CI 1.22-1.86, p=0.0002) and the use of Busulfan in the conditioning regimen (HR 0.30, CI 0.16-0.58, p=0.0004). Using age (≥50 years) and the absence of Busulfan in the conditioning regimen as risk factors, the incidence of severe aGVHD was 0%, 26% and 58% in patients with no, one or both risk factors, respectively. Overall survival at 5 years in the same three groups was 74%, 62% and 30%, respectively (p=0.004).

Conclusion: In this study, we found that patient age, as expected, increased the risk of severe aGVHD while Busulfan in the conditioning regimen decreased this risk. One explanation to our findings may be that most patients in the Busulfan group also got ATG, and this combination had an additive effect on immunosuppression.

P507

Long-term survival follow-up and factors associated to response and survival of patients treated with daclizumab for steroid-resistant acute graft-versus-host disease

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Steroid-resistant acute Graft-Versus-Host-Disease (aGVHD) following allogeneic hematopoietic stem cell transplantation (HSCT) is associated with a high mortality rate and has no standard treatment. The aim of our retrospective study was to report the long-term outcome of patients treated by daclizumab, a humanized monoclonal antibody targeting the α chain of the IL-2 receptor, for seroid-resistant aGVHD, and to identify prognostic factors associated with response to treatment and survival. Twenty patients with grade II to IV steroid-resistant aGVHD were included between 1997 and 2004. All had hematologic malignancies and 12/20 were in an advanced phase of the disease. The donor was match related (n=14), MUD (n=2) or mismatch UD (n=4). 7/20 had a RIC. All patients received daclizumab 50 mg/d I.V. daily for 7 days than twice a week than once a week for a median duration of 27 days (5-150). Daclizumab was given second line after steroid failure in 15 patients and 3rd line in 5. The median number of injections was 10 (5-35). Daclizumab was clinically well tolerated although 65% developed episodes of infections during or following therapy. The complete response rate of aGVHD was 20% at day 8 and 25% at day 28. Survival rate at 1 year and at 5 years are 35% and 25% respectively, with a median survival time of 86 months. Overall, 15 patients died in this cohort. The causes of death were aGVHD (n=7), relapse (n=4), prolonged pancytopenia (n=2), pulmonary cGVHD (n=1) and infection (n=1). In univariate analysis, an advanced phase of the disease at time of HSCT was associated with a poor response rate at day 8 or day 28 and had also a negative impact on survival with 62% survival for early phase patients compared to 18% survival for advanced phase (P=0.03). The strongest predictive factor for survival was the response to daclizumab at day 28 which was associated with a 67% probability of survival compared to 9% for non responding patients (P=0.0024). In conclusion, daclizumab can be a reasonable option for the treatment of steroid-resistant aGVHD since it provides an acceptable response and long-term survival rates. The disease status at transplant is the only clinical factor at time of HSCT associated with response rate and overall survival. Interestingly, infections are not the first cause of death. Further prospective studies are needed to determine the best treatment schedule and to identify patients who are more likely to benefit from this approach.

P508

Successful treatment of ulcerating chronic cutaneous GvHD after allogeneic SCT with split-skin allografting from the stem cell donor: a case report

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Objective: Treatment of acute and chronic Graft-versus-Host Disease (GvHD) still belongs to one of the most challenging problems after allogeneic stem cell transplantation (SCT). The development of extensive chronic GvHD of the skin is frequently associated with pain, immobilisation due to sclerosis and strictures and a high risk for secondary infections in case of ulcerations.
Patient: We report about a 29-year old woman who received a peripheral blood stem cell graft after myeloablative conditioning from her HLA-identical sister for Flt3positive AML in 08/04. GvHD prophylaxis consisted of CsA and MTX. One month after the engraftment the patient developed acute GvHD grade IV of liver and gut, which was successfully treated with sequential combinations of 6 immunosuppressive drugs; finally in 06/05 immunosuppression could be tapered and stopped. In 09/05 chronic cutaneous GvHD was diagnosed which became rapidly severe affecting the whole integument in a lichenoid and sclerodermiformic manner which required de novo systemic immunosuppression with Prednisolone and Tacrolimus (FK). Despite 3 different treatment lines cutaneous GvHD progressed resulting in extensive ulceration of the scalp (12x17 cm) until 06/08. As conservative treatment did not result in re-epithelialisation and was complicated by relapsing secondary infections, we planned an allogeneic split-skin grafting from her HLA-identical sister. The first step of reconstitution consisted of wound debridement and a first meshed skin graft covering 100% of the ulceration under continous immunosuppression with Prednisolone, FK, MMF and MTX. After three months 50% of the wound was covered by engrafted donor-skin and the other 50% could be covered in a second step of skin transplantation after wound debridement.
Results: This procedure resulted in a complete healing of the large ulcerated area within 5 months. Interestingly,following the donor skin graft we were able to gradually taper systemic immunosuppression from MTX, FK, MMF and Prednisolone to MMF alone without local or systemic deterioration of GvHD.
Conclusion: Split-skin transplantation from a HLA-identical sibling donor represents a treatment option for severe therapy-refractory ulcerating chronic GvHD. Absence of GvHD in the grafted skin demonstrates tolerance and suggests chronic GvHD to be mediated by a continuing alloreaction. Whether donor skin grafts exert a systemic immunomodulating effect remains speculative and needs further investigation.

P509

Graft manipulation for prevention of graft-versus-host disease in paediatric non-malignant diseases

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Background: Donor T-cells have a therapeutic potential in patients transplanted for malignant diseases, however are of no major purpose in non-malignant diseases. Aim of the study:
1. to prevent gvhd by CD34+-selection or CD3/CD19+-depletion or both;
2. to facilitate engraftment by high doses of purified CD 34+-PBSCs and/or to retain engraftment helper cells by CD3/CD19+-depletion.

Patients and methods: 13 patients, median age 9.9 years, were treated: 6 with severe aplastic anemia (SAA, 5 of them refractory to previous immunosuppression), 2 with Hurler´s disease and 1 each with Wiskott-Aldrich-syndrome, Krabbe´s disease, Kostmann´s disease, Fanconi anemia and severe combined immunodeficiency, respectively. Eight PBSC-donors were unrelated, of whom four were 12/12, two 11/12 and two 10/12 HLA matched. Of the 5 family donors 2 were matched siblings, two the haploidentical fathers and one the 8/10 HLA-matched mother.11 patients received reduced intensity conditioning; ATG or OKT3 was used in 13, thiotepa in 9, fludarabine in 8, cyclophoshamide in 8, melphalan or treosulfan in 2, total lymphoid irradiation in 2 and busulfan in 1 patient. Median 2 PBSC-aphereses were run. CD34+-selection and /or CD3/CD19+-depletion was performed on two consecutive days with the Clinimacs system. Median yield of purified CD34+number was 12.9x10e6/kg (4.93-139) and median CD3+cell dose was

5.8x10e4/kg (0.85-24.5). Products were either transfused shortly after the manipulation (5 patients) or cryopreserved, and transfused later (8 patients) because of delays of the transplants for clinical reasons. Immunosuppression was none in 5 patients and cyclosporine or mycophenolate mofetil up to day + 60 in the others. Results: All patients engrafted. Leucocyte engraftment >1000/ul was median 10 days (8-17) and platelet engraftment >30.000/ul 12 days (10-19). Ten patients survive with a median of 78 months (7-152) with stable engraftment and a median of 98,53% chimerism (90,65-100) on day 100. None developed gvhd greater than grade 2. Two patients rejected and died 9 and 13 months later, respectively; one patient died from CMV-pneumonitis on day + 33.
Conclusion: CD34+-selection for the treatment of non-malignant diseases may provide high numbers of CD34+-cells for rapid engraftment and prevention of gvhd. CD3/CD19+-depletion, which retains engraftment helper cells, may additionally facilitate engraftment and hasten immunologic recovery.

P510
Steroid refractory intestinal GvHD treated with ileocecal resection
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A 42 years old male patient with a myelofibrosis received according to the EBMT-Study for myelofibrosis (N. Kröger, BMT 2009) a reduced intensify conditioning with busulfan 6,4 mg/kg BW and fludarbine 180 mg/m². The HLA-indentical graft (PBSC) was from his sister. GVHD prophylaxis was done with antilymphocytes globuline (ATG Fresenius), cyclosporine A and mycophenolat mofetil (MMF).
On day +50 MMF was stopped. On day +118 with the reduction of cyclosporin A a skin-GVHD grade III (Glucksberg criteria) was confirmed with a biopsy. It showed a good response to a standard treatment with methylprednisolon (MP) 2 mg/kg. During the tapering of MP acute watery diarrhea with nearly 2000 cm³/day occured. Clinical presentation, gastrointestinal endoscopy with a gut biopsy confirmed an intestinal GVHD grade III. MP was rised again to 2 mg/kg without a response. A salvage treatment with tacrolimus plus basiluximab, an anti CD-25 antibody, sirolimus, budenosid and MMF was also failing. An ultrasound examination of the abdomen showed an wall thickening mainly in the distal small bowel. MR enterography and ballon assisted enteroscopy cofirmed a presentation of a intestinal GvHD as terminal ileitis. A wall edema and inflammatory lesions were detected only in the last part of the ileum. Biopsies were taken in all parts of the small bowel and colon. Apoptotic changes associated with a GVHD were seen only in the terminal ileum. After an ileocecal resection with ileocolonic anastomosis a remission could be achieved. On day +300 after transplantation the treatment was continued with tacrolimus and MP 20 mg/day. Diarrhea was not observed.
In this case a steroid refractory intestinal GVHD could be treated by a surgical intervention with success. The ileocecal resection was indicated after proving a segmental presentation of a GVHD with MR imaging and endoscopic examination of the whole small bowel. Both diagnostic methods are useful in severe intestinal GVHD. They could support the decision for a resection of parts of the gut to cure a GVHD. MR enterography is although useful in grading of intestinal GVHD parallel to histological and clinical criteria.
Pictures: 1. MRI, 2. Endoscopy, 3. Histology, 4. Ileum during the surgery.

P511
Transplant outcomes are not affected by CMV serostatus in patients with acute graft-versus-host disease
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Bone marrow transplantation (BMT) is an accepted treatment for patients with a variety of haematological malignancies, bone marrow failure syndromes and immunodeficiencies. CMV infection is a major cause of morbidity and mortality after allogeneic transplantation. Recently, the survival difference between patients at risk for CMV reactivation and those who were not, was found to be similar. However, the impact of GvHD in patients with different CMV serostatus regarding transplant outcomes remains unknown.
Materials and methods: We retrospectively analysed 196 patients who underwent an allograft over a ten year period. Of these, 68 patients had GvHD (12 grade III-IV) and 121 patients had no GvHD. 76 patients were transplanted for myeloid malignancies and 111 for lymphoid and 9 were transplanted for bone marrow failure syndromes. 111 had a sibling donor and 85 had an unrelated donor. 76 patients were not at risk for CMV reactivation and 44 of those didn't develop GvHD. 35 patients were low risk (CMV donor pos/recip neg) and of these, 20 had no evidence of GvHD. 85 of them were at high risk of CMV reactivation (CMV donor pos/recip pos or donor neg/recip pos) 57 of these patients had no GvHD.
Results: The median age was 47 years (range 17-66 years), the median follow up was 30 months (range 1-127 months) and the 10 year overall survival 48%. All patients engrafted. The transplant related mortality was 23%. In our cohort of patients with GvHD and different CMV serostatus the overall survival was 53%, corresponding to the lower number of relapsed patients compared to the non GvHD cohort. However a higher transplant related mortality was noticed amongst this cohort compared to patients with no evidence of GvHD. Our standard first line treatment for GvHD was steroids and our second line Infliximab for patients who didn't respond to steroids.There was no difference in overall survival between patients who were at risk for CMV reactivation with GVHD and those who were not at risk for CMV reactivation and developed GvHD.
Conclusion: In accordance with recent literature CMV status did not have any adverse impact on overall survival in allogeneic transplant patients. In addition, this work shows for the first time the presence of GvHD did not impact on survival, regardless of CMV serostatus. This may be due to efficient pre-emptive CMV treatment and regular monitoring of CMV levels in the blood. However this needs to be studied in a large cohort of patients.

P512
Non-myeloablative transplantation with or without T-cell depletion in unrelated donor stem cell transplantation
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Background: Nonmyeloablative conditioning hematopoietic stem cell transplantations (NST) induce engraftment of allogeneic stem cells with a low spectrum of toxicity, but graft-versus-host disease (GVHD) remains a significant cause of morbidity and mortality. In vivo or in vitro T-cell depletion have been used to reduce the incidence of GVHD. However, these may have an adverse impact on disease response due to amelioration of graft-versus-tumor effect.
Patients and methods: To evaluate the efficacy of types of T-cell depletion, we compared the results in 26 patients with T-cell depletion using unrelated donor stem cells. 25 patients were acute leukemia and one patient was myelodysplastic syndrome. Conditioning regimens consisted with fludarabine based busulfex (n=20), melphalan (n=3), low dose TBI (n=1) and cyclophosphamide (n=2). 16 patients were received

alemtuzumab (n=12) or antithymocyte (n=4. GVHD prophylaxis were with FK506 with or without short course methotrexate (n=22) and cyclosporine with or without methotrexate (n=4).
Results: There were no significant differences in sex, age, status at transplantation, total infused CD34+ cells and CD3+ cells number between two groups. In patients underwent T-cell depleted transplantation, there was a tendency to increase of graft failure and rejection in non-manipulated group (31% vs.10%, p=0.57). CMV disease after transplantation occurred in three patients, all of whom received T cell depleted transplantation. There were no differences in the incidence of acute GVHD(≥ Gr II) and chronic GVHD(limited and extensive) between two groups (p=0.84, p=0.52 respectively). Chimerism studies at day 14 and 28 indicated that the majority (65%) attained complete donor chimerism and there was no difference in proportion of complete donor chimerism at day 14 and 28 (p=0.75). With a median follow-up of 175 days (range, 41-1618), event free survival at 1 year was 70% for non-manipulated group and 50% for T-cell depleted group (p=0.23). No difference was observed in overall survival at 1 year between two groups (65% vs. 39%, p=0.72) but there was a tendency of prolonged survival in non-manipulated group conferred to lower incidence of graft rejection and relapse after transplantation.
Conclusions: In summary, T-cell depletion with NST using unrelated donor stem cells didn't show any benefits in the incidence of acute and chronic GVHD but its use was associated with a higher incidence of CMV disease and graft rejection.

P513

Patients suffering from graft-versus-host disease develop severe organ siderosis – Case report and retrospective analysis

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Introduction: Iron overload (IO) leads to damage of parenchymal organs. Recently, it was demonstrated that IO prior allogeneic stem cell transplantation is correlated with increased mortality. While the IO is contributed to transfusions of erythrocytes, we found transfusion-independent severe signs of organ siderosis during the course of graft-versus-host disease (GvHD) in humans and in a murine model of GvHD. In this study, we present one patient with GvHD and clinical and histological signs of organ siderosis without excessive transfusions and analyse retrospectively the development of organ siderosis during GvHD.
Methods: For our retrospective analysis, patients after allogeneic transplantation and surviving longer as 60 days were included in our analysis. In total, 101 patients were assessed. Routineously assessed ferritin, bilirubin, transferring, C-reactive protein, leucocytes, iron values and the GvHD scores were assessed.
Patient: A 29 year old female patient was allogeneic transplantated due to a severe aplastic anemia with leading thrombopenia without significant anemia. 2 years prior transplantation, she received 2 packages of erythrocytes, during aplasia after transplantation she needed only 2 more packages. Around day +150 the patient developed a mixed GvHD. The laboratory values indicated a organ siderosis. This was confirmed in histological liver and bone marrow sections. Furthermore, a myocardial hypertrophy was found. Treatment of GvHD resulted in prompt improvement of both the laboratory values and the clinical performance. A relapse of GvHD was again accompanied by signs of iron overload, and again treatment could resolve these findings.
Retrospective analysis: In our analysis, 24 of 101 patients developed severe signs of organ siderosis after allogeneic stem cell transplantation, determined by laboratory values and partial by histological findings, especially bone marrow histologies. The organ siderosis was associated with the occurrence of graft-versus-host-disease and a worse overall prognosis.
Conclusions: Some patients develop organ siderosis while suffering from graft-versus-host-disease independent from erythrocyte transfusions. Treatment of GvHD improves the signs of iron overload. While the effect and treatment of iron overload prior allogeneic transplantations is well described, the occurrence of iron overload during GvHD is not well understood. Whether iron chelation during this phase is beneficial is to be investigated.

P514

Brazilian experience in extracorporeal photopheresis for treatment of acute and chronic graft-versus-host disease

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Background: Extracorporeal Photopheresis is a cellular therapy that has demonstrated efficacy in treatment of graft-versus-host disease after hematopoietic stem-cell transplantation.
Objective: To demonstrate the safety and efficacy of extracorporeal photopheresis with UVADEX® in patients with acute and chronic GVHD after HSCT unresponsive to first-line therapy.
Methods: Prospective study conducted in the bone marrow transplant center (CEMO) of the Instituto Nacional do Câncer (INCA) in Rio de Janeiro, Brazil, from November 2000 to December 2010. Were included twenty-nine patients with chronic GVHD and nine with acute GVHD. It was used Therakos XTS system and UVADEX® (methosalen) in extracorporeal photopheresis procedure.
Results: It was considered complete response, withdrawal of immunosuppression or complete clinical resolution; and partial response, reduction of immunosuppression or at least 50% improvement in clinical picture.Among patients with chronic GVHD, twenty-five were classified as having a severe form and the rest as a moderate. In these patients, seven (24%) achieved complete response and sixteen (55%) achieved partial response.
Overall response: 75% (twenty three individuals).
Among patients with acute GVHD, four had grade III and five had grade IV disease. Four patients had involvement of skin and liver and five had involvement of skin, liver and intestine. In these patients, complete response was achieved in five (55%). The response was not evaluable in the other four due to early death.
Adverse events: in patients with chronic GVHD, seven had catheter obstruction (in total, eleven patients had central venous catheter), one developed anemia, one had photophobia and one had herpes zoster; in patients with acute GVHD, one had catheter obstruction (all patients had CVC), two developed intestinal bleeding, four had hypotension, one had tachycardia and hypothermia and two had sepsis.
Conclusion: Extracorporeal photopheresis seems to be safe and effective as a second line treatment for acute and chronic GVHD. The use of this therapy in steroid-resistant patients should be considered as an earlier therapeutical approach especially in those with acute disease.

P515

High-dose cyclophosphamide for GvHD prophylaxis after HLA-matched or haplo-identical allogeneic haematopoietic stem cell transplantation: a single-centre experience

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Introduction: recent studies reported that after unmanipulated bone marrow transplant (BMT) from HLA-matched1 or haploidentical (haplo) related donor2, GVHD prophylaxis with post-transplant cyclophosphamide (pt-Cy), respectively given alone or with Tacrolimus (FK-506) plus Mycophenolate Mofetil (MMF), was associated with low incidence of graft rejection and acute

and chronic GVHD (aGVHD, cGVHD) in patients (pts) with advanced myeloid malignancies (AMM).
Methods: in our centre, 7 pts with AMM (1 CML in blast crisis, 1 CML in accelerated phase, 3 AML not in CR, 2 AML in CR2 relapsed after autologous BMT) underwent T-cell replete BMT from HLA-matched (2) and haplo (5) donors. GVHD prophylaxis was performed with Cy 50 mg/kg at days (d.) +3 and +4, alone or with FK-506 and MMF in HLA-matched and haplo BMT, respectively. In the haplo BMT, the conditioning regimen (Cond) was Cy 14.5 mg/kg at d. -6 and -5, fludarabine 30 mg/m² from d. -6 to -3 and TBI 200 cGy at d. -1 in 4 pts; in a patient Cy was substituted with thiotepa 5 mg/kg at d. -6 and -5. In HLA-matched BMT, Cond was fludarabine 120 mg/m² and busulphan i.v. 12.8 mg/kg in 4 days.
Results: the median time to reach ANC>500/microl was 17 d. (r. 13-26) in 7/7 of pts, and platelets>30000/microl was 28 d. (r. 19-32) in 6/7 of pts. After a follow-up of 11.5 months (m.) (r. 2-21), 4 pts are alive in CR (haplo and HLA-matched BMT in 3 and 1 case, respectively). Two pts (28%) developed steroid-responsive grade 2 cutaneous aGVHD at d. +18 and +39. After FK-506 and MMF withdrawing, 2 pts undergone haplo BMT had delayed grade 2 intestinal GVHD, responding to standard therapy. Interestingly, Foxp3 levels were significantly low in pts developing aGVHD. None of our pts showed cGVHD. CMV reactivation was documented and cured in 71% of pts.
A patient (12.5%) died for transplant-related complications at 2 m. while 3/7 (43%) relapsed and 2/7 (28%) died after 4.3 and 8.5 m. Both these pts had refractory AML at the transplant and received BM from haplo donors.
Conclusions: pt-Cy is an effective prophylaxis of aGVHD and cGVHD in HLA-matched or haplo allogeneic BMT. This approach is safe with low toxicity and low incidence of severe complications. To reduce relapse rate, especially after haplo BMT in pts with active disease, we are proposing clofarabine and busulfan i.v. as Cond in a multicenter study of GVHD prophylaxis with high-dose Cy after allogeneic BMT.
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2. Kasaman. BBMT 2008;14:641-51.

P516

Extracorporeal photopheresis for the treatment of acute graft-versus-host disease in patients after allogenic haematopoetic stem cell transplantation

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Introduction: Acute graft-versus-host disease (aGVHD) is one of the most common and severe complications of allogeneic hematopoetic stem cell transplantation (allo-HSCT). Patients with aGVHD not responding to treatment with steroids have a poor prognosis. Extracorporeal photopheresis (ECP) is used for the treatment of chronic GVHD in patients after allo-HSCT. The efficacy of ECP in the treatment of aGVHD has to be determined.
Material and methods: Ten patients (pts) with steroid-refractory (SR) aGVHD were included in the study. Clinical characteristics of the patients who underwent ECP: acute myeloid leukemia -4, acute lymphoblastic leukemia -4, chronic myeloid leukemia-1, Fanconi anemia-1; median age at ECP, years (range) 17,8 (3-39) ; median interval GVHD-ECP, days (range) 22,3 (5-70); median interval allo-HSCT-ECP, days (range) 53,8 (23-109); myeloablative conditioning regimen-7, reduced intensity conditioning regimen-3; cyclosporine A-based prophylaxis of aGVHD- 7, tacrolimus-based – 3. Glucksberg grading system was used for assessment of aGVHD. Seven allo-HSCT recipients received ECP as a second-line and 3 allo-HSCT recipients received ECP as a third-line therapy. Two of the patients had Grade I acute GVHD (isolated aGVHD of skin stage 2 in both pts), 5 pts had Grade III aGVHD (2 pts with isolated skin involvement, 3 pts with multisystem involvement) and 3 pts had Grade IV aGVHD (1 pt with isolated gut GVHD and 2 pts with multisystem involvement). In overall 9 pts had aGVHD with skin involvement, 4 pts had gut involvement and 2 pts had liver involvement. ECP treatment was performed on once a week schedule using “off-line” technique (Cobe Spectra-Macogenic).
Results: Complete response (CR) or partial response (PR) was achieved in both pts with Grade I GVHD. In Grade III patients CR or PR was achieved in 4 pts of 5 pts, a non-responding pt was with concurrent hepatitis C, later died of hepatic failure. In Grade IV GVHD response was not achieved in 2 pts, one pt completely responded to ECP as a third-line therapy. Complete responses in the skin were in 4 pts (56%), gut – 2 pts (50%). Partial responses in the skin were in 3pts (33%). Overall non-responses were in 3 pts (30%).
Conclusion: ECP can be used as treatment of steroid refractory aGVHD with maximal response in skin aGVHD.

P517

Small-scale extracorporeal photopheresis for treatment of chronic graft-versus-host disease: feasibility and evidence for dose-response

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Objective: Chronic Graft-versus-Host Disease (cGvHD) is the main cause of long-term morbidity and non-relapse mortality after hematopoietic stem cell transplantation (HSCT). Primary treatment of cGvHD relies on steroids. Conventional extracorporeal Photopheresis (ECP) has proven its efficacy, steroid-sparing potential and safety as second line treatment, but in children requires a double lumen central venous access (CVL). Here we describe the use of small scale ECP (ssECP) in a pilot patient and report dose-response observed.
Patient and methods: The 18 year old female patient had been transplanted from a 9/10 HLA-compatible matched unrelated donor for secondary myelodysplastic syndrome after immunosuppressive therapy of severe aplastic anemia. The preparative regimen consisted of thiotepa, fludarabine, treosulfan; GvHD prophylaxis was anti-thymocyte globuline, cyclosporine A and methotrexate. Acute GvHD of the skin and gastrointestinal tract was successfully treated with steroids. Severe cGvHD of skin, liver and mucous membranes was treated with steroids and mycophenolic acid. cGvHD remained unchanged and ECP was considered. As the patient had venous access problems but refused a double lumen CVL, ssECP was started 11 months after diagnosis of cGvHD. GMP-compliant ssECP was performed by drawing 100 ml of blood twice a week. After centrifugation in a closed system, mononuclear cells (MNC) were isolated according to Hackstein et al. (Transfusion 2009) with minor modifications. MNC were incubated in 200–220 ng/ml 8-methoxypsoralen and irradiated with 2 J/cm² UV-A (Maco-Genic). A mean of 57 ± 22 x 10^6 MNC was reinfused per procedure.
Result and conclusion: Skin GvHD and joint mobility improved slightly after initiation of ssECP, liver enzymes were still increasing. Seven weeks after the beginning of ssECP prednisone therapy had to be tapered due to drug-induced diabetes mellitus. ssECP was intensified to 5 procedures per week with a product from 109 ± 21 ml autologous blood, followed by 3 procedures in the next week and tapered with 2 sessions weekly with a product from 141 ± 54 ml blood. At the time of intensification, we saw a substantial improvement of the skin and joint manifestations and a definite decrease in liver enzyme values. Taken together, we were able to implement the ssECP procedure safely and demonstrate that it can be effective in the treatment of cGvHD. A high initial frequency with daily ssECP might be needed to gain best response.

P518

Acute graft-versus-host disease outcome during a 6-year period: a retrospective analysis

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Objectives: Acute graft-versus-host disease (aGVHD) remains one of the main causes of morbidity and mortality following allogeneic stem cell transplantation (SCT). Patients(pts) who failed to respond to steroids as first-line treatment of aGVHD have usually poor outcomes. There is no standard therapy uniformly accepted for refractory aGvHD.
Methods: We retrospectively reviewed our experience analyzing 22 pts (13 males, 9 females) treated for aGVHD between August 2005 and September 2010.
Results: The median age was 34 years (range 5-62) and grafts were from HLA matched siblings (8), matched (5) or mismatched unrelated donors (7), haploidentical (1) or cord blood donors (1).
Indications for SCT were acute leukemia (14), CML (1), myelodysplasia (2), myelofibrosis (1), lymphoma (2), thalassemia (1) and SCID (1) in early (9) or advanced status of disease (13). Stem cell source was peripheral blood in 5 pts, bone marrow in 15, both in 1 and cord blood in 1. The conditioning regimen was myeloablative in 20 pts and reduced intensity in 2 pts. Most pts received cyclosporine (CSA) and MTX with (19) or without (1) methylprednisolone (MP) and 2 pts received CSA + MP as initial GVHD prophylaxis. ATG was part of the preparative regimen for 53% of pts. The diagnosis of aGVHD was established at a median of 37 days (range 11-201) after transplantation and confirmed by biopsy in 42% of the cases. AGVHD grade I-II was 32%. Grade III-IV occurred in 68% of pts and was more frequent in unrelated recipients (11) compared to related siblings (3). Initial treatment consisted of MP up to 2 mg/kg in 10 pts and 3 to 5 mg/kg in the remaining.The overall complete response (CR) to first-line was observed in 5 pts (22%). Second-line treatment was pentostatin alone or plus other in 9 pts, cyclophosphamide, mycophenolate, infliximab in the others. Overall 5 pts (23%) responded to the second-line (CR 14%, PR 9%). The pts who failed to respond received further salvage therapies including infliximab, rituximab, pentostatin or extracorporeal photopheresis. All refractory pts died without response. Fifty% of the responding versus 0% of the non-responders survived (4 pts died from relapsed or progressive disease, 9 from GVHD, 4 from infections, and 1 from organ failure).
Conclusions: Systemic steroids are the main therapy for aGVHD, but treatment failure is common and the outcomes remain very poor. It is still very important to find new methods of GVHD treatment. Our retrospective analysis confirms this need.

P519

Can we recognize clinical parameters which are associated with occurrence and severity of graft-versus-host disease?

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The application of allogeneic hematopoetic stem cell transplantation (HSCT) is limited by life-threatening complications such as severe or acute graft-versus-host disease (GVHD). Despite intensive prophylaxis with immunosuppressive agents, the incidence of GVHD occurs in 9-50% of patients undergoing transplant with an identical HLA sibling matched donor and 75% of patients undergoing unrelated HLA donors.
Aim of study: To evaluate our experiences in GVHD prophylaxis and treatment after alloTHSC, GVHD incidence and prognostic factors and administration of new immunosuppressive regiments.
Patients and methods: Starting from September 2000 till September 2009, 63 patients (36 males and 27 females) at the age of 16-56 (median range 33 years) with hematological malignances were treated with alloTHSC on Department of Hematology, Clinical Centre, and Skopje. For 55 patients donors were HLA identical siblings and the rest 8 patients were transplanted from HLA unrelated donors. In 10 patients bone marrow was used as source of stem cells and in 53 patients stem cells were obtained from peripheral blood. From the group of 63 patients, 26 patients have active disease at the time of transplantation. GVHD prophylaxis was accomplished with combination of cyclosporine and metothrexate (Seattle regimen) or more intensive immunosupression regiments.
Results: GVHD was noticed in 30 patients (47,6%) and at 33 patients (52,4%) was not noticed a manifestation of GVHD. Acute GVHD was noticed in 24 patients (38%) and chronic GVHD in 20 patients (31,7%) The remaining 32 patients (45%) achieved complete clinical and hematological remission. Lethal outcome was confirmed in 31(49%) patients (9 from chrGVHD,6 from acute GVHD, 16 from disease relapse).
Conclusion: The incidence of acute GVHD in our study was 38% and 31% of chronic GVHD. The most common GVHD reaction was registered in female donors and male recipients, with higher GVHD incidence in elderly patients. In all patients stem cells were obtained from peripheral blood. Active disease, sex, source of hematopoetic cells, age and conditional regiments are the most significant predictive factors with the higest influence of incidence of GVHD.

P520

Graft-versus-host disease management

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Graft versus host disease (GVHD), donor stem cells in patients with an immunological reaction caused by the healthy T-lymphocytes. Risk factors for GVHD, HLA mismatch, age, sex mismatch, underlying disease, type and stage, and is used in prophylaxis. GVHD allo BMT (bone marrow transplantation), after the most serious complication that can occur. The disease is usually the skin, gastrointestinal tract and liver effects, however, lungs, eyes, muscle-skeletal system, also affect the vagina and vulva. Acute GVHD and chronic GVHD graft versus host disease is divided into two.
1 - Acute GVHD.
2 - Chronic GVHD:
- Skin: erythema, dryness, itching
- Nail: Fracture
- Hair: alopecia and premature bleaching
- Eye: Dryness, Itching
- Vulva and vagina: vaginal dryness and atrophy
- Liver: impaired liver function tests
- Lung: Pulmonary function tests impaired
- Mouth: Dryness, mucositis
- Gastrointestinal System: Nausea, vomiting, diarrhea
- Musculoskeletal system: joint pain
- Hematological findings: severe thrombocytopenia
- Immune deficiency: recurrent infections, sinusitis.

As a result of GVHD to treatment-related or patients: skin, hair, nail problems, skin care, because, mucositis assessment and oral care, nutrition because of the lower and upper gastrointestinal problems, fluid-electrolyte balance is important. Immune insufficiency due to the risk of infection due to symptom control and prevention measures must be taken. Need to follow through due to dryness and itching eyes. According to laboratory tests, and symptom control organs is required. Vulva and vagina can occur due to problems of change seksüalitede. Cortisone is used in treatment of diabetic patients may develop due care, such as osteoporosis exercise programs due to be established. Physical integrity because of the disease and the treatment of patients with body image distortion and shock may develop psychological problems should be addressed. Our

center and our approach to evaluation of GVHD are shown below. Should be checked at regular intervals during follow-up programs GVHD underwent significant changes or increased frequency of follow-up and maintenance.

P521
Single-centre experience GvHD
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Graft versus host disease (GVHD), donor stem cells in patients with an immunological reaction caused by the healthy T-lymphocytes. Risk factors for GVHD, HLA mismatch, age, sex mismatch, underlying disease, type and stage, and is used in prophylaxis. GVHD allo BMT (bone marrow transplantation), after the most serious complication that can occur. The disease is usually the skin, gastrointestinal tract and liver effects, however, lungs, eyes, muscle-skeletal system, also affect the vagina and vulva.
Materials and methods: Between June 2001 and June 2009 in the Adult Hematology, Hacettepe University Faculty of Medicine after allogeneic stem cell transplantation (AKHN) during assessment of patients with GVHD symptoms, treatment, treatment complications, and responses were reviewed retrospectively. Obtained from the percentage calculation and chi-square tests were used in the evaluation of the data.
Results and discussion: According to the data of the study, 92 patients AKHN 90.2% of the 'cine reduced intensity (RIC), 9.8% of the full regimen used in allogeneic stem cell transplantation. Cyclosporine as GVHD prophylaxis regimens to prepare patients, 3 mg/kg^{-1}. day was started and metotoraxat 1 days and 10 mg/m^2, 5 mg/m^2 on Day 3, 6 days were given 5 mg/m^2. The level of graft and renal function after the drug was given according to the form of oral cyclosporine. AKHN of these patients in 64.2% of acute leukemia, 14.1% 'i AA, 10.9% and 10.9% of lymphoma u' u other diseases (MDS, CML, PNH, Myelofibrosis) constitute a group. Patients, 58.7% male, 41.3% third of women. 53.7% of men and percent female donors, 57.9% of female patients' u are male donors. Looking at the rate of 96.7% and HLA antigens in groups of full match donors' dir. 18% of patients with chronic GVHD, 10.1% in developed acute GVHD. 64.7% of patients with chronic GVHD 'have limited liability, geçirenlerin acute GVHD in 55.6% of grade 1 skin period.

Stem cell source

P522
Bone marrow may be the preferable graft source for transplant recipients homozygous for HLA-C group 2 ligands for inhibitory killer Ig-like receptors
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Acting as ligands for inhibitory killer cell Ig-like receptors (KIR), HLA class I molecules participate in natural killer (NK) cell regulation. One individual may express inhibitory KIR to which it lacks a corresponding HLA ligand. The role of this "missing KIR ligand" constellation in hematopoietic stem cell transplantation (HSCT) remains controversial, depending on incompletely defined transplant variables. We have retrospectively analyzed the effects of missing HLA-C group 1/2 and Bw4 KIR ligands on the outcome in 382 HSCT, comparing 118 bone marrow transplants (BMT) to 264 peripheral blood stem cell transplants (PBSCT). In the multivariate Cox analysis of PBSCT, poor progression-free survival (PFS) was observed for recipients homozygous for HLA-C group 2 (C2/2) (risk ratio [RR], 1.59; p=0.026). In contrast, C2 homozygosity was not unfavorable after BMT (RR, 0.68; p=0.16). C2 homozygous recipients (n=68) had better PFS after BMT than after PBSCT (RR, 0.17; p=0.001), due to fewer relapses (RR, 0.27; p=0.018). Missing Bw4 favorably influenced PFS after BMT (RR, 0.56; p=0.04), but not after PBSCT. For the first time these data demonstrate differential effects of missing KIR ligands in BMT versus PBSCT. Since C2 homozygous recipients fared poorly after PBSCT, BMT should be (re)considered for these patients.

P523
Intra-bone cord blood transplant is associated with reconstitution of haematopoietic stem cell reservoir in adult patients
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In this study we show the reconstitution of hemopoietic progenitor cell reservoir in 50 adult patients transplanted intra-bone with UCB (Umbilical Cord Blood) cells with a follow-up of at least three months.
All patients included in this study achieved a complete hematological reconstitution and were in remission of their disease for the entire duration of the study.
Clonogenic progenitors (CFU-GM, BFU-E, CFU-GEMM) reached a frequency within normal value at day + 30 and did not show any subsequent quantitative variation during the entire investigation period (from day +30 to 3 years). The CFU-F (colony-forming-cell Fibroblast) frequency was very low (median 1/10^{6}MNC, range=0-16) and did not improve with time. Soon after transplant some MSC were found of donor origin but this population rapidly declined (from 20% at day +100 to 3% at 1 year). LTC-IC frequency was systematically studied in 28 patients with sufficient number of time-points up to 1 year: overall LTC-IC frequency was rather low for long time after CBT. However, this study identifies two groups of patients: (1) patients who did not achieve normal LTC-IC frequency (15-75/10^6 MNC) at any time points and (2) patients who achieved normal LTC-IC frequency in at least one point. In particular,LTC-IC frequency after one year post-CBT correlated with the number of TNCx10^7/kg (Spearman's rho=0.01) and with the number of CD34+x10^5/kg (Spearman's rho=0.05). Interestinghly, patients injected with high cell and CD34+ number show a constant increasing LTC-IC frequency. In the other patients the detection of LTC-IC was variable and inconsistent with time; this finding may suggest a non homogeneous distribution of progenitors within the marrow areas. Parallel analysis showed that LTC-IC frequency in patients transplanted with adult hematopoietic sources (bone marrow and mobilized peripheral blood) remains at very low levels (0-3/10^6 MNC)up to 10 years post transplant.
In conclusion, we demonstrate here, for the first time in adult patients, that the combination of an immature stem cell source (CB) and the intra-bone route of administration may allow the full reconstitution of the stem cell (LTC-IC) pool when a "sufficient" number of stem cells are infused.

Table =LTC-IC/10^6MNC at different time point from IB-CBT

		day +30	day +100	6 months	1 year	1.5 year	2 years	3 years
Group 1	median(range)	0 (0-6)	4 (0-11)	0 (0-9)	0.2 (0-9)	2(0-12)	2 (0-5)	2.5 (0-5)
p=		**NS**	**NS**	**0.01**	**0.02**	**NS**	**0.02**	**0.01**
Group 2	median(range)	0 (0-6)	2 (0-19)	3 (0-16)	9.4 (0-72)	10 (0-50)	15 (0-91)	30 (10-70)

P524

CD34 cell dose predicts chronic graft-versus-host disease incidence whereas CD3 cell dose and day 56 chimerism predicts survival in non-myeloablative allogeneic peripheral blood haematopoietic cell transplantation

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Purpose: Compared to myeloablative regimens NMA HCT is associated to a decreased toxicity but higher incidence of cGVHD and relapse. We studied the influence of peripheral blood stem cell (PBSC) graft composition, engraftment kinetics and post-transplant chimerism on outcomes in multiple myeloma patients treated with a tandem approach.
Methods: We retrospectively analyzed graft composition and chimerism data in a homogenous cohort of 81 consecutive patients treated for multiple myeloma with HLA-matched sibling NMA HCT after a FluCy based conditioning. All patients underwent a high dose Melphalan based autologous transplant followed by a NMA allogeneic HCT in a tandem approach. GVHD prophylaxis included tacrolimus and mofetyl mycophenolate. FDC was defined as ≥95% donor CD3 circulating cells.
Results: Progression-free (PFS) and overall survival (OS) at 8 years was respectively 42,7% and 70,7%. All but 8 patients achieved FDC at day 180. Median time from transplantation to FDC was 120 days. Four out of the 8 patients with persistent mixed chimerism (MC) experienced progression, 2 of them before day 100. Graft composition in CD3 and CD34 cells were correlated (r=0.291, P=0.009).CD34 cell dose <5 $x10^6$/kg was associated to a lower incidence of cGVHD (73% vs 92% p=0,039). CD3 cell dose < $25x10^7$/kg was associated to an improved PFS (53% vs 13%, p=0,004). Day 100 chimerism did not predict incidence of cGVHD, relapse, or overall survival. Day 56 chimerism was higher for patient who experienced aGVHD (median 89% vs 78%, p=0,048). Day 56 chimerism <82.5% was associated to an improved OS (84% vs 53%, p=0,01).
Conclusion: Limiting CD34 cell dose in PBSC graft could lower the incidence of cGVHD presumably without impacting relapse incidence and overall survival. Limiting CD3 cell dose in PBSC graft could improve survival. Early evaluation of chimerism at day 56 is relevant to identify patients who will experience aGVHD and can predict survival.

P525

Impact of initial volume and initial nucleated cell count on mononuclear and CD34+ cell efficiency recovery after umbilical cord blood unit processing

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Umbilical cord blood (UCB) is an alternative hematopoietic stem cell (HSC) source for recipients lacking a suitable donor. Initially restricted to pediatric patients, UCB is now increasingly used as a source of HSC for adults as well, a fact that increases the pressure to deliver processed units with a high cellularity. Though several studies investigated whether events upstream of processing such as mother- or birth-related factors could be predictive of unit cell content, the identification of processing parameters associated with a high cell recovery remains a priority.
We report here our results on volume reduction of 1553 UCB units collected in the maternity wards of 5 distinct swiss counties over a 10 year-period, processed using hydroxyethyl-starch induced sedimentation with an automated device, under routine laboratory conditions.
Mean initial volume, total nucleated cell (TNC), mononuclear cell (MNC) and CD34+ cell (CD34C) counts of collected units were respectively 127±30 ml, 1.51±0.55E9, 0.68 ±0.38E9, and 4.8±3.4E6 (±SD). Mean TNC, MNC and CD34C processing efficiencies (PEf) were 77±10%, 85±11%, and 79±12% respectively. TNC and MNC, but not CD34C PEf were significantly decreased when processing units contained <2.05E9 cells. When plotted against initial volume, TNC PEf remained stable up to <170 ml and decreased thereafter. By contrast MNC and CD34C PEf followed a bell-shaped curve with a maximum for initial volumes of 150 and 130 ml respectively. Waste bag content analyses uncovered a positive correlation between unit initial volume and the fraction of TNC and MNC seeding in the waste. The effect of the delay between UCB collection and processing on TNC and MNC PEf was also investigated. Delays >25 hours were associated with a significant decrease of TNC and MNC PEf, independently of initial volume and TNC counts ($p<0.05$). Moreover TNC viability in processed unit negatively correlated with time delay ($p<0.007$).
Altogether our data show that initial unit volume exerts a strong effect on PEf. Thus the best yield for CDC34C is expected with units of initial volumes ranging from 110 to 170 ml, processed within 24 hours after collection. Splitting units prior to processing should be envisaged only when initial volume is >180 ml because units <90 ml also exhibit a poor recovery of CD34C. Hopefully these observations may help producing therapeutic units with the highest cell number that could be offered to a large panel of patients.

P526

CXC chemokine receptor 4 gene polymorphism affects the haematological recovery after transplantation of peripheral blood progenitor cells

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Objectives: The CXCL12/CXCR4 axis plays a critical role in the mobilization, migration, survival and homing of CD34 cells. Our previous studies on the CXCL12 (3'UTR-801 G>A) gene polymorphism showed the significant association of the donor CXCL12-3'A allele with the higher efficacy of G-CSF induced mobilization of CD34 cells (Bogunia-Kubik et al. 2009) and faster recovery of granulocytes and platelets in the recipients of autologous peripheral blood progenitor cells (PBPC) (Gieryng et al. 2010). The present study aimed to analyse the effect of the CXCR4 gene polymorphism (C>T substitution) on the hematological recovery after PBPCT reflected by the granulocytopoiesis (expressed as the day post transplant when the number of granulocytes exceeded 500/ul) and megakaryopoiesis (day of platelet count $>20x10^3$/ul).
Methods: Fifty-seven autologous and 106 allogeneic patients and their donors were investigated for association between the pace of haematological recovery and the number of CD34+ cells in the graft. The CXCR4 genotyping was performed in 45 autologous patients and 83 donors donor-recipient pairs of allogeneic PBPC transplants with the use of PCR-RFLP.
Results: In both autologous and allogeneic settings the correlation between the pace of granulocyte and platelet recovery ($p<0.001$ and $p<0.005$ after autologous and allogeneic PBPCT, respectively). In allogeneic PBPCT recipients granulocyte recovery was faster in patients transplanted with sibling as compared to alternative donors ($p=0.046$) and correlated with the number of transplanted CD34 cells ($p=0.045$). Recipient CXCR4-T allele was found to be associated with faster recovery of platelets (mean: 12 vs. 17; $p<0.001$). The multiple regression analysis (considering: patients' age and sex, number of transplanted CD34 cells, CXCR4 SNP) confirmed the independent association of the CXCR4-T allele ($p=0.044$) with platelet recovery after allogeneic PBPCT.

Conclusion: These results show that CXCR4 gene polymorphism contributes to the haematological recovery after allogeneic PBPCT.
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P527
Enhanced survival and thymic reconstitution in patients with non-malignant diseases after cord blood transplantation
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We performed a retrospective single-center analysis to evaluate umbilical cord blood transplantations (UCBT) including thymic reconstitution.
Between 2001 and 2010, 50 UCBT were performed. Of these, 37 were single and 13 double UCBT. The study included 30 children and 20 adults. Eighteen patients had non-malignant diseases and 32 had hematological malignancies. Myeloablative conditioning was given to 34 patients and 16 received reduced intensity conditioning (RIC). All patients received ATG. The majority of children received a 5/6 HLA matched graft and adults 4/6. In 31 patients T cell receptor excision circle (TREC) levels were evaluated with real-time PCR at 3, 6, 9, 12 and 24 months. Mesenchymal stem cells (MSC) were given to 9 patients, 7 as prophylaxis, one due to hemorrhagic cystitis and one due to GVHD.
The total nucleated cell dose was 4.4 (1.4-100) x 10^7/kg. Engraftment was seen in 87% of the patients (n=46), and median time to engraftment was 29 days (3-79). Complete donor chimerism in all cell lineages was shown in 59% (n=37) at day 100, and 68% (n=22) at 1 year. Rejection was diagnosed in 7 patients (14%). Overall survival (OS) was 55% at one year and 46% at five years. There was a statistically significant difference in OS between patients with non-malignant and malignant disease (p=0.026), at five years 72% and 28%, respectively. Transplant-related mortality at 100 days and one year was 16% and 30%, respectively. In patients with hematological malignancies, the cumulative incidence of relapse was 40%. The cumulative incidence of acute graft-vs-host disease (GVHD) grades II-IV was 34%, and grades III-IV was 16%. In multivariate analysis, factors associated with acute GVHD grades II-IV were major ABO mismatch (p=0.05), ATG dose >6.5 mg/kg (p=0.012) and RIC (p=0.047). Chronic GVHD was diagnosed in 12% of the patients. Patients given MSC had significantly less survival 11% vs 63% (p=0.03). Surprisingly patients treated with supportive MSC therapy also showed a significantly worse TREC recovery. In multivariate analysis patients receiving MSC (p=0.001) and low CD34 dose (p=0.0026) had significantly lower TREC levels whereas children had significantly higer TREC levels (p=0.001).
In conclusion, UCBT was associated with good results in patients with non-malignant diseases. Thymic reconstitution is enhanced in children, patients with non-malignant diseases and patients receiving a high cell dose but worse in patients given MSC after UCBT.

P528
The role of G-CSF combination in conditioning regimen for CBT patients with AML
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Study purpose: While we have performed almost 7000 cord blood transplantation (CBT) in Japan, those results are quite heterogeneous. Among acute myeloid leukemia (AML) patients, event-free survival at 5 years was 63% in the Institute of Medical Science, University of Tokyo (BBMT 14: 1341, 2008), however, leukemia-free survival at 2 years was 36% in the registration data (Blood 113: 1631, 2009). Based on the results of ex vivo (BMT 13: 239, 1994) and animal model (Nat. Biotech 28: 275, 2010) studies, we have developed and used granulocyte-colony stimulating factor (G-CSF) combined with cytarabine (Ara-C) in the conditioning regimen to increase the susceptibility of Ara-C mediated cytotoxicity. To determine the role of G-CSF combination in conditioning regimen for AML patients, we have analyzed registration data in Japan.
Patients and methods: Clinical data of 676 patients (age:16-55 years) with AML who received CBT without prior transplant history between 1998 and 2008 in Japan were collected by the Japan Cord Blood Bank Network. The median period of follow-up for survivors (n=349) after transplants was 23 (range, 1-122) months. We analyzed the hematopoietic recovery, risk of relapse, overall survival (OS) and disease-free survival (DFS) using competing risk regression models.
Results: OS at 5 years of all patients was 43% and those myeloablative conditioning regimen (MAC, n=545) and reduced-intensity regimen (n=128) were 47% and 22% When we focused in patients received MAC, OS at 5 years of patients using high-dose total body irradiation (TBI>10Gy, n=470), low-dose TBI (<10Gy, n=26) and no irradiation (n=44) were 52%, 34% and 37%. MAC with high-dose TBI consisted of 3 groups, TBI + Ara-C + cyclophosphamide (CY) [Group-1], TBI +Ara-C combined with G-CSF +CY [Group-2], TBI +others [Group-3]. Then, we analyzed 438 AML patients using MAC with high-dose TBI and calcineurin inhibitor plus methotrexate method. In multivariate analysis, G-CSF-combined regimen significantly contributed the beter engraftment of neutrophil and platelet. OS (p=0.0874 for Group-1 vs -2 and p=0.0487 for Group-2 vs -3), relapse rate and DFS (p=0.0442 for Group-2 vs -3) in Group-2 tended to be better and some of them indicated significant difference. Those results at 3 years of each group were 56%, 70%, 46% and 37%, 18%, 27% and 40%, 64%, 39%, respectively.
Conclusion: The G-CSF-combined conditioning regimen promotes better engraftment and survival results in CBT for AML patients.

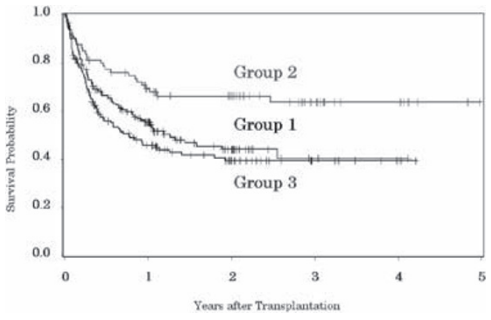

Figure. Disease free survival.

P529
Impact of prior autologous mobilization status on engraftment and outcome after allogeneic stem cell transplantation for lymphoma and myeloma
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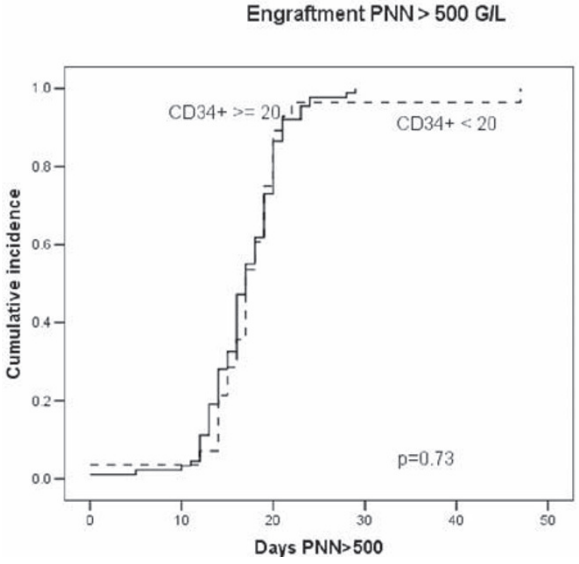
Approximately 15% of patients who are candidates for autologous HSCT fail to collect an adequate amount of CD34+ cells (poor mobilizers). Reasons for poor mobilization include patient and treatment-related factors. Infra-clinical damages to the bone marrow (BM) micro-environment may play a role and affect the interactions of stem cells with stromal cells and the bone-blood barrier that contribute to efficient mobilization. If such a hypothesis stands true, then damages to the BM micro-environment should also affect the ability of donor stem cells to home and seed in the BM of recipients after allogeneic HSCT. We thus examined whether patients who underwent allogeneic HSCT at

a single institution, using fludarabine-busulfan-ATG RIC conditioning after a failed attempt at autologous HSCT, were different in terms of hematopoietic recovery than patients who received a tandem autologous and allogeneic HSCT.
Inclusion criteria were age > 18 years, diagnosis of lymphoproliferative disease or multiple myeloma, prior mobilization (succesfully or not), HSCT from related or unrelated donor and RIC conditioning. Poor mobilization was defined as circulating CD34+ <20/µl on the first day of planned collection. PMN and PLT engraftment were determined for good and poor mobilizers, taking into account confounding factors such as age, type of donor and disease.
A total of 148 patients were included in this analysis, of which 125 were evaluable for CD34+ counts and engraftment (n=33 for poor-mobilizers and 92 for good mobilizers, see table 1). All but two patients engrafted. Overall, the median (range) day was 17 (0-47) for PMN >0.5 G/L, 8 (0-49) for PLT >20 G/L and 12 (0-49) for PLT >50 G/L. There was no significant difference in PMN (figure 1) or PLT engraftment between the two subsets. Nevertheless, NRM and overall mortality were higher in poor mobilizers (p=0.009 and p=0.01 respectively); relapse rate, acute and chronic GvHD were similar in both groups (p=0.81). Data were confirmed after adjustment for patient's age, type of donor and disease.
Our data suggest that lymphoma and myeloma patients with a history of poor mobilization defined as CD34+ <20/µl do not have different outcomes in terms of PMN and PLT engraftment. Despite the absence of difference in terms of early hematopoietic recovery after allogeneic HSCT, NRM and mortality were significantly higher in poor mobilizers.

	Total	*CD34 < 20/µl*	*CD34 >= 20/µl*	*p*
Patients	148	33	92	
Bu/Bx 1 day	1	0	1	0.59
Bu/Bx 2 days	133	31	81	
Bu/Bx 3 days	13	2	10	
Bu/Bx 4 days	1	0	0	
ATG 1 day	69	15	45	0.22
ATG 2 days	69	16	41	
ATG 3 days	7	0	5	
ATG 4 days	5	2	1	
HLA-identical sibling	116	23	75	0.25
Unrelated donor, 10/10	23	8	13	
Unrelated donor, 9/10	6	2	3	
Haploidentical donor	1	0	1	
Hodgkin's lymphoma	13	3	10	**0.02**
Non-Hodgkin's lymphoma	73	21	40	
Chronic lymphocytic leukemia	12	3	1	
Multiple myeloma	50	6	41	
PBSC	133	29**	84*	0.73
BM	15	4	8	
Median age	52 (21-68)	53 (24-66)	51 (25-63)	0.32
* n=3 cryo ** n=1 cryo				
Median (range) PMN > 500		17 (0-47)	17 (0-29)	0.62
Median (range) PLT > 20		10 (0-40)	8 (0-38)	0.24
Median (range) PLT > 50		13 (0-44)	13 (0-49)	0.96



P530
Haplo-identical stem cell transplantation for the non-Caucasian population
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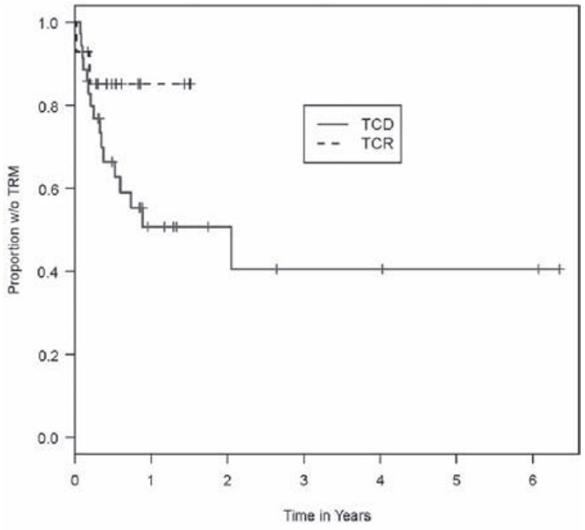
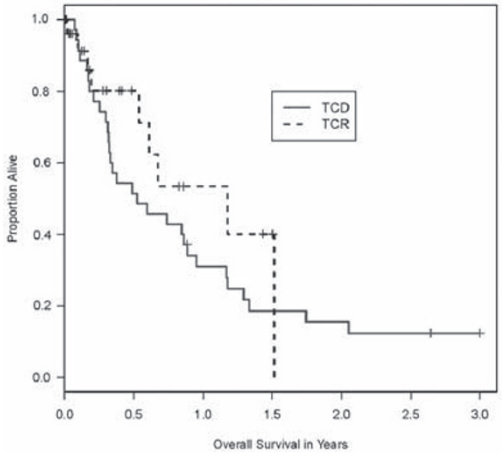
Hematopoietic transplantation has been performed mostly from matched related or unrelated donors (MUD). It has been difficult to identify a MUD for non-Caucasian patients (pts) in the Registries. Of all 2117 pts transplanted at MD Anderson past 25 years, 1677 (79.2%) were Caucasians (C), 271(12.8%) Hispanics (H), 109 (5%) African-Americans (AA) and 33 (1.5%) Asians (A). Similar racial distribution was noted with patients receiving a 9/10 MUD during the same period of time. We hypothesized that haploidentical stem cell transplantation (HaploSCT) would be an alternative for the non-Caucasian population without a matched donor and treated 24 pts with a T-cell replete graft and conditioning regimen consisting of fludarabine (40 mg/m²/day x 4), melphalan (100-140 mg/m²) and thiotepa (5-10 mg/kg). GVHD prophylaxis was accomplished with high-dose





post-transplant cyclophosphamide (50 mg/kg/day x 2), tacrolimus and mycophenolate.
Results: Racial distribution in this group was: 8/24 (33.3%) C, 6/24 (25%) H, 5/24 (21%) AA, 4/24 (16.6%) A, 1/24 (4%) other, for a total of 66.6% non-Caucasian population. Median age was 47 years (24-65). All patients but one received bone marrow stem cells. 4 pts had prior allogeneic transplants. 13 pts had AML/MDS (8 poor-risk cytogenetics), 6 pts CML/MPD (5/5 blast phase CML), 5 pts lymphoma/CLL. Donor-recipient HLA matching was: 12 (50%) were 5/10, 3/25 (12%) were 6/10, 5/25 (20%) were 7/10 and 4/25 (16%) were 8/10. 10 pts (42%) were in remission at the time of transplant. All 23 evaluable pts (one had early death) engrafted, all but one with 100% donor chimerism after a median of 19 days (5-40). Cumulative incidence of day-100 treatment-related mortality (TRM) was 14%. No patients less than 50 years died of TRM. Grade II-IV aGVHD occurred in 4 patients and cGVHD in 1 patient. 7/18 (39%) pts relapsed while only 1 for the pts in remission at the time of transplant. After a median follow-up of 6 months (range 3-18) for survivors of > 100 days (N=14), OS for the whole group was 80%, while PFS for pts in remission at the time of transplant was 89% (N=10, CI 43-98%). No differences in OS/PFS were noted between the Caucasian and non-Caucasian population. These results are better for TRM and OS than our retrospective data with T-cell depleted HaploSCT (Figures).
Conclusion: HaploSCT is a feasible and safe transplant alternative for the non-Caucasian population without a matched donor.

P531
Intrabone cord blood transplant: preliminary results from a prospective phase II study
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Intrabone transplantation has been described as an efficient way to infuse cord blood. Here we report on the first 10 patients undergoing to intrabone transplantation for advanced haematological malignancies. The study was a phase II prospective monocenter study approved by local ethical Committee and registered at http://clinicaltrials.gov/; the primary endpoint was engraftment rate and 17 patients are planned. All patients signed a written informed consent. End of recruitment is planned within April 2011.
Conditioning regimen was myeloablative (Bu- Cy or unfractioned TBI-Cy); prophylaxis of GVHD was CsA, micophenolate 30 mg/kg/die and ATG-F 30 mg/kg, total dose. G-CSF was routinely given from Day +7 until recovery.
CB processing was as described by Frassoni et al. Briefly, cord blood units were thawed, washed with the Rubinstein solution to remove DMSO and reduced the final volume up to 30 ml.
Infusion was performed in operating room using a monitored anaesthesia care sedation with propofol and remifentanil.
Median age was 36 years (29-54), median weight of recipient was 60 kg (51-93); diagnosis were AML (7) ALL (1) MM (1) CML (1). Phase at transplant was mainly advanced (for AML 5 with resistant/relapsed disease, 2 II CR; for ALL 1 II CR; MM: 1 progressive disease and CB for CML). 3 patients had a previous allotransplant and 3 a autotransplant. All CB units were 4/6 except for one (5/6).
Median total cell infused was 1.91 x 10e7/kg and median CD34 pos cells 0.52 x 10e5/kg.
Median time to 0.5 x 10e9/L ANC was 21 days and median time to 20x 10e9/L and 50 x10e9/L platelets were 46 and 60.5 days, respectively. At day +100 the evaluable patients had 121x 10e9/L plt (range 104-192). Two patient died before engraftment for CNS bleeding (+6) or for myocarditis (+16).
One patient didn't graft and a second unit, again via intrabone, was given, after 2 months and with a RIC regimen, with successful engraftment.
All the evaluable patient achieve a complete response (CR), except for the MM pt, who obtain a nCR); two patients relapse at 4 and 7 months from transplant.
GVHD occurrence was very low: no severe acute GVHD and only one case of extensive GVHD were recorded.
Preliminary results of this study with advanced disease suggest that intrabone injection of CB resulted in short term good engraftment, expecially for platelets, low GVHD and good outcome. Longer follow up is needed to estimate the actual antileukemic effect.

P532
Volume reduction with HES improves haematopoietic progenitors function after cord blood thawing
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Background: Volume reduction and cryopreservation are critical steps in umbilical cord blood (UCB) banking that determine the quality of stored units. Some methods have been described for volume reduction as red blood cell (RBC) sedimentation with hydroxyethyl starch (HES, 1.5%), semi-automated methods as top and bottom and automated systems.
Aims: Our objective was to evaluate the quality of UCB units volume reduced with and without HES, after cryopreservation and thawing, and to assess the factors that can influence hematopoietic progenitors functionality.
Methods: We reviewed data from 994 cord blood units cryopreserved in Valencia Cord Blood Bank. Volume reduction was performed by HES sedimentation (n=219) or top and bottom fractionation without HES (n=775). Cryopreservation was performed in a controlled manner (1-3°C/min) using automatic devices. A satellite cryovial was thawed from each cord blood units for biological tests: cell count, viability and clonogenic assays (CFU) were performed.
Results: After volume reduction mean ± S.D. for total nucleated cells (TNC), CD34+ cell and htc were 105.44 ± 34.18x 10^7, 37.64 ± 27.60 x 10^5, 17.58 ± 10.30% for HES group, and 115.16 ± 33.76x10^7, 46.52 ± 32.67x10^5 and 39.17 ± 7.64%, respectively ($p<0.05$ for all variables). Correlation among TNC and htc before cryopreservation and after thawing was significant ($p<0.001$). Mean TNC recovery, htc and CFU after thawing were 93.12 ± 7.79%, 17.09 ± 4.89% and 128.73 ± 107.63 x 10^4 for HES group, and 90.36 ± 11.67%, 39.06 ± 8.74% and 83.92 ± 48.73 x10^4 for non HES group ($p<0.05$ for all variables). Results of clonogenic assays by microliter and viability according to the volume reduction methodology are shown in the following table. When comparing HES data according to HTC ≤ 17 and >17, clonogenic assays and viability were similar. However, when comparing no HES group according to htc ≤ 40% and >40%, there was statistical difference for viability (76.87 ± 10.13% vs 73.24 ± 11.66%, p=0.000), platelets (404.74 ± 136.54 x 10^6/ml vs 462.20 ± 154.33 x 10^6/ml, p=0.002) and CFU-GM (20.54 ± 15.41 x 10^4 vs 18.39 ± 11.29 x 10^4. p=0.010).
Conclusions: Presence of HES in cryopreservation solution (less than 1%) improves the hematopoietic progenitors function after cord blood thawing. For those cord blood units cryopreserved without HES, higher hematocrit and platelets reduces viability and functionality of progenitors.

Volume reduction	CD34 cell /microliter	BFU-E /microliter	CFU-GM /microliter	CFU-GEMM /microliter	Viability %
HES (n=219)	77.68 ± 56.89	13.62 ± 12.10	26.51 ± 22.15	1.21 ± 1.30	78.47 ± 8.89
No HES (n=775)	100.55 ± 79.61	10.62 ± 6.09	17.93 ± 10.34	0.65 ± 0.80	75.24 ± 10.10
p	0.01	0.00	0.00	0.00	0.04

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Comparison between two automatic devices for cord blood volume reduction

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Background: Volume reduction of cord blood (CB) units is a proceeding used in most cord blood banks in order to reduce red blood cell content and cryogenic space. To date, many methods have been described for this purpose as red blood cell (RBC) sedimentation with hydroxyethyl starch (HES), semi-automated methods as top and bottom and automated systems. Automatic devices SEPAX and AXPTM have been specifically developed for CB volume reduction. In the SEPAX the UCB-HES program requires addition of HES to the CB unit, while AXP consists of a microprocessor-controlled device and disposable closed bag set and do not require HES addition.
Aims: Our objective was to compare two different automatic devices used for volume reduction in routine CB banking.
Methods: AXP: Cord blood was transferred to the bag set and centrifuged in the AXP device for 30 minutes. The device separates the three layers of CB into different bags during the two centrifugation steps. 2. SEPAX: The collection bag (cord blood plus HES) was attached to the processing kit, the kit was installed on the machine (programme CB-HES). All CB units were volume-reduced to less than 30 ml. Volume and cell counts were determined before and after volume reduction process.
Results: We processed 1000 UCB units with AXP and 669 with SEPAX. Final volume and red blood cell depletion were 24.61±3.64 ml and 84.75±5.40% for AXP and 24.06±1.30 ml and 84.79±7.62% for SEPAX respectively (p=0.000 and p=0.002). Final hematocrit was 26.36±3.76% for AXP and 24.00±9.09% for SEPAX (p=0.000). Results of cell recoveries are shown in the following table.
Conclusions: SEPAX system provides higher total nucleated cell recoveries and RBC depletion than AXP, while hematopoietic progenitors (measured as CD34+ cells) recovery was similar. AXP system achieves acceptable cell recoveries and high red blood cell depletion without HES.

Device	Cell recoveries (%)			Viability (%)
	TNC*	Lymphocytes	CD34+ cells	
AXP (n= 1000)	73.11 ± 7.51	71.48 ± 13.35	95.14 ± 28.62	89.72 ± 6.7
SEPAX (n=669)	75.65 ± 6.95	71.46 ± 12.7	98.07 ± 26.99	90.19 ± 5.41
p	0.000	ns	ns	ns

*Total nucleated cells

P534

CD34+ cell content as criteria for selecting umbilical cord blood units for cryopreservation and storage

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Background: Umbilical cord blood (UCB) has become an important source of hematopoietic progenitor cells for unrelated donor bone marrow transplantation. Cord blood banking activities has decisively contributed to this fact. Most UCB banks require a total nucleated cell (TNC) content of at least 60 x 10^7 to 140 x 10^7 for cryopreservation and storage. The CD34+ cell content has shown to have influence on engraftment and survival of UCB transplants. Valencia Cord Blood Bank determines the CD34+ cell content before processing in order to select the UCB units for cryopreservation and storage. The final objective is to increase the UCB units quality.
Aims: Our objective was to analyse the impact of CD34+ cell content as selection criteria for cord blood unit cryopreservation and storage, in adition to TNC cell content. Methods: CD34+ cells were quantified by flow cytometry to UCB units containing TNC ≥ 100 x 10^7. Cord blood (5 x 10^5 cells) was incubated using monoclonal antibodies conjugated with CD34 fluorescein and CD34 phycoerythrin and 7-aminoactinomycin D as marker of DNA staining. Flow cytometric analysis was performed with computer software CellQuest.
Results: Our Bank has determined CD34+ cell content to 1737 cord blood units containing TNC ≥100x10^7 along a two-year period. Only UCB units containing CD34+ cells ≥20x10^5 were finally cryopreserved and storage. Applying this approach has led to an improvement in the UCB quality. Mean and S.D. TNC and absolute CD34+ cells have increased from 104.60 ± 35.36x10^7 and 31.15 ± 29.93x10^5 (n=1968) to 108.79 ± 34.17x10^7 and 38.61 ± 40.07x10^5 (n=1910), respectively. Number of UCB units according to TNC and CD34+ cell content are shown in the following table.
Conclusions: Introducing CD34+ cell content as selection criteria increases the UCB refusal rate, but also the cell content and quality of UCB units.

	TNC x 10^7			
CD34+ cells x 10^5	>= 100 n=1737	>= 120 n= 1277	>= 140 n=831	>= 160 n=546
> 20	1553	1187	791	526
> 30	1245	1019	706	488
> 40	978	827	600	436
> 50	714	617	472	356
> 60	540	477	381	297

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High cellularity in discarded filters after bone marrow collection

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Introduction: After bone marrow harvest, collected cells are filtered to remove fat, bone fragments and clots from the final products which will be infused into the recipient. Together with discarded materials, a certain amount of hematopoietic cells are kept into the filter. We analyzed discarded filters in order to assess the amount of cells which are lost by means of filtration.
Patients: Cell contents were analyzed in 35 bags with the corresponding filters which were harvested for 19 patients; 17 were allogeneic donors (4 unrelated and 13 related) and 2 were candidate for autologous transplantation. Of 35 discarded filters, 28 were left over at the end of bone marrow harvest filtration in the operatory room, while 7 were left over after infusion at bed-side.
Methods: Discarded filters were washed with normal saline in the Laboratory to recover cells trapped in; counts of residual cells were made by using the cell coulter Coulter Act.diff, Beckman coulter®. Cells entrapped in the filters were used to produce mesenchymal stromal cells.
Results: The number of cells counted in the filters varied, with a median of 233x10^6 cells (range 6-2000) overall, 285x10^6 cells (range 6-2000) in the “operatory room” filters and 48x10^6 cells (range 18-95) in the “bed-side” filters. The number of discarded cells was not correlated with the nucleated cell concentration in the bags, the total number of nucleated cells, nor the collected volume in the original bag. Cells entrapped in the filters showed a high potential for mesenchymal stromal cell (MSC) production, with 1.8x10^9 MSC obtained after expansion in platelet lysate out of 300 mononucleated cells.
Conclusions: Cell dose has been shown to play a crucial role in bone marrow transplantation. A relevant amount of cells is lost by filtration, with an higher loss at first filtrations in the operatory room compared with subsequent ones at bed-side. The variability of cell content may suggest that a better procedure for filter washing may decrease the amount of cell loss. Cell characterization by flow cytometry may provide additional data.

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Standardization and automation of the colony-forming cell assay for measuring haematopoietic progenitors in cord blood

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The colony forming cell (CFC) assay is the standard functional assay for measuring the hematopoietic progenitor content of cord blood (CB) and other cell products for hematopoietic stem cell transplantation (HSCT). Automation of colony enumeration and shorter assay duration would improve the utility of the CFC-assay. To this end we developed an imaging and analysis instrument (STEMvision™) for automated colony enumeration and classification and evaluated two CFC-assay formats: 1) the regular 14-day assay in MethoCult H4034 medium for enumerating erythroid, myeloid and multilineage colonies, and 2) a novel 7-day assay in MethoCult Express medium for detecting total progenitors in CB without distinction of colony types. We examined the accuracy and reproducibility of the 7 and 14-day assays and compared colony counts scored manually by different laboratory staff members or counted automatically using the STEMvision instrument. Manual colony counts of 7 and 14 day CFC-assays on CB correlated strongly (R2=0.97) and were, on average, <10% different from each other (slope =0.97 (95% confidence limits: 0.94-1.00), n=36). Manual and automated colony counts of 7-day CFC-assays also correlated strongly (R2=0.98) with differences <10% (slope=0.96 (0.92-1.00), n=32). High correlation was also observed between manual and automated counts in 14-day assays (R2>0.8, n=39), while automated counts of total, erythroid and myeloid colonies were, approximately 10-20% higher than the respective manual counts. The coefficient of variation (CV) of colony counts in >30 cultures counted manually by 3-4 different people was 12%, 11% and 17% for total, erythroid and myeloid colonies, respectively. The CV of STEMvision results of replicate images of the same cultures was 5%, 10% and 9%, demonstrating that automated colony counting is at least as reproducible as manual colony counting by experienced people. Automated colony scoring by STEMvision provides an accurate and reproducible method to enumerate hematopoietic progenitors in CB. The shorter, 7-day, assay and automated colony scoring will provide quicker results and increased standardization to evaluate graft quality in CB banking, cell processing and HSCT. Additional optimization of the automated counting method is expected to further increase the agreement between manual and automated CFC counts. STEMvision should also be easily adapted to automated counting of colonies from peripheral blood and bone marrow.

P537

Volume reduction of bone marrow using an automated, closed system

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Objectives: This study aims to highlight the effectiveness of an automated technique, for volume reduction of stem cells from bone marrow. This system allows to manipulate blood units in a closed loop in order to comply with the GMP criteria. Also, purchase a considerable importance, especially in the case of transplantation with ABO incompatibility. The critical importance of cell dose in the clinical outcome has motivated the need to develop techniques aimed at reducing cell losses and increasing data reproducibility. The aim of this study is to evaluate the efficacy of the Sepax S-100 (Biosafe Group, Eysins, Switzerland) device to process volume reduction HPC-M. In particular, we have analysed nucleated cell (NC), depletion red blood cells (RBC), volume reduction and CD34+ cells recovery.
Methods: 27 procedures were carried out on concentrated collected from 21 patients, undergoing autologous transplantation. The “Volume Reduction” protocol is designed to remove RBC and plasma, while maintaining a good recovery of mononuclear cells. The processing protocol of sample from 30 to 880 ml, relies solely on the hematocrit value and the initial volume of the sample to be processed, to calculate the final volume. To evaluate CD34+ cells the protocol ISHAGE was used.
Results: In the patients who underwent autologous transplant, the automated procedure resulted in a median recovery of 80% (58-109; ±DS 13.1) NC and 89% (59-134.9; ±DS 22) CD34+ cells, depletion RBC 73% (81-62; ±DS 5.41); volume reduction 88% (93-85; ±DS 2.2). In In the patients who underwent allogeneic transplant, the automated procedure resulted in a median recovery of 75,1% (55-98; ±DS 11.8) NC and 79.6% (59-158; ±DS 24) CD34+ cells, RBC depletion 75.4% (82-62±DS 5.5); volume reduction 88% (91-83; ±DS 2.4).
Conclusion: The volume reduction of bone marrow using the Sepax S100 automated and closed system, does not affect the cell functionality assuring a satisfactory result in terms of nucleated cells, CD34+ cells recovery, RBC depletion and volume reduction. The results showed a recovery of NC and CD34 + equal to 80%, RBC depletion to 75%, without the use of HES, a reagent commonly used in the absence of an automated procedure, but relying solely on the hematocrit value and the initial volume of the sample to be processed.

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Unfrozen autologous haematopoietic stem cells transplantation in myeloma patients

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Usual autologous HSC transplant requires a priming chemotherapy followed by granulocyte growth factor to harvest the peripheral CD34+ cells. These latter are freezed in DMSO solution and stored frozen until they’re reinfused into the patient after a conditioning second chemotherapy course. The freezing is required to span the time from the priming and the conditionig courses. Plerixafor proved an efficient mobilising agent without a chemotherapy priming, enabling to start the conditioning soon after the HSC harvest. In this setting, when the conditioning lasts only one day, the cell freezing is not needed, and the reinfusion will be performed without DMSO. To explore the feasibility of such a schedule we treated 4 myeloma patients who underwent a mobilisation with filgrastim+plerixafor. In all cases a previous mobilisation with chemotherapy+filgrastim failed and the patients were successfully mobilised with plerixafor according to the producer (Genzyme Co) guidelines. They were 3 males (51, 66, 66 y) and a female (65 y), all received only a single dose of plerixafor but a male (66 y) who needed 2 doses, and were submitted to a single apheresis. Soon after the collection they were conditioned with a single dose of melphalan 200 mg/Kg and after 24h were reinfused with the harvested cells, stored at + 4 C°. They received 4.7; 2.25; 2.73 and 5.82 $x10^6$/Kg CD34+. The engraftment data have been compared with those of a cohort of 20 myeloma patients treated in the last three years, 12 males and 8 females, median age 60 y (range 47-70),who received frozen/thawed autologous HSC. All transplants engrafted. The four study patients reached 0.5 x 10^3 neutrophils/µL on day +10; +10; +10; +9; 20 x 10^3 platelets/µL on day +10; +13; +13; +10; and 50 x 10^3 platelets/µL on day +15; +19; +24; +10. In the control cohort the patients received a median of $4.35x10^6$/Kg CD34+ cells (CI: 3.36-5.34; range 1.25 - 16.58) the median engraftment time was:for neutrophils day +12 (CI: 11.42-12.58; range 8-13), for 20 x 10^3 platelets:

day +14.5 (CI: 11.61-17.4; range 7-31); for 50 x 10^3 platelets day + 21 (CI: 12.92-29.08; range 10-40). Our data show that the reinfusion of autologous HSC unfrozen, a day after the harvesting, is safe, with an engraftment probability not inferior to the thawed cells, and possibly allows better platelets recovery. This schedule avoids the DMSO toxicity both for the patient and for the cells, and the cost and damage of freezing, storing and thawing the cells.

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The intra-bone cord blood transplant experience in Genoa: the engraftment is not influenced by the time of cryopreservation or from the bank of origin of cord blood units

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In this study we compared the qualitative and quantitative data of 80 samples of cryopreserved cord blood from accredited banks which we received for intra-bone transplants.
The samples were divided in 4 groups according to the time elapsed between the collection/freezing and thawing. This parameter (time) was compared with: (a) the number of total nucleated cells (TNC), (b) the total number CD34+ cells, (c) the time of engraftment (PMN and PLT) as described in the table. No statistically significant differences were found among the 4 groups (Mann-Whitney test).
Furthermore, it was evaluated whether the cell product quality could be influenced by the areas of the CB bank of origin subdivided as: (a) extra-European banks (55%), (b) European (20%) and (c) Italian (20%) The quality of the examined samples was studied as (i) viability, (ii) CD34 + cell recovery and (iii) in vitro progenitor cells growth (CFC= colony forming unit). Overall, median value of viable cells was 70% for all banks; median TNC number was 160,25 x 10^7 (range of 67.1–415); CD34 + cell recovery was : median 7.17 x 10^4 (range 1.8–25.79); progenitor cells grew a median of 260.34 x 10^4 (range 2.24–1200.7). The origin of the Bank did not influence any of these variables including time of engraftment (PMN and PLT) (data not shown. This fact implies high standards provided by all the Banks in the cryopreservation and harvesting of cord blood units. Moreover, the transport in dry-shipper for long periods (concerning extra-European banks) had no influence on the quality of units once thawed.

YEARS	N° Samples	NC x10^7		CD34$^+$ x10^5		PMN (DAYS)		PLT (DAYS)		P
		Median	Range	Median	Range	Median	Range	Median	Range	
0-2	21	160,00	83.40 - 403	5.27	1.8 - 18.9	22	15-33	32	22-62	N.S
3-5	27	160,00	85 - 318	6.90	2.16 - 20.55	23	14-39	34	16-63	N.S
6-8	17	144,00	67.10 - 415	8.03	2.52 - 8.03	23	15-44	36	28-70	N.S
9-11	15	178.18	91.2 - 275	7.34	3.35 - 15.84	24	15-36	34	26-64	N.S

P540

Efficacy of automatic DMSO washing in autologous stem cells transplant

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Objectives: To date, peripheral blood stem cells (PBSC), in autologous haematopoietic stem cell transplantation, are the preferred source of hematopoietic stem cell in respect to the bone marrow and cord blood due to several advantages including more a rapid engraftment. The critical importance of cell dose in the clinical outcome has motivated the need to develop techniques aimed at reducing cell losses and increasing data reproducibility. The aim of this study is to evaluate the efficacy of the Sepax S-100 (Biosafe Group, Eysins, Switzerland) device to process thawed HPC-A products. In particular, we have analysed viability, nucleated cell (NC), hematopoietic progenitors (CFU-tot) and CD34+ cells recovery.
Methods: Forty-three procedures were carried out on concentrated collected from 34 pediatric patients. The "PBSC-Washing" protocol is designed to remove the DMSO after thawing. The protocol processing of sample from 50 to 150 ml and allows it to thaw two bags. To evaluate the CD34+ cells and viability "The protocol of the International Society of Hematotherapy and Graft Engineering (ISHAGE)" was used. The samples were incubated with anti-CD34+ phycoerythrin-conjugated and anti-CD45 fluorescein isothiocyanate-coniugated (BD Bioscences, San Jose, CA, USA) monoclonal antibodies. Cell vitality was tested using 7-aminoactinomycin D (7-AAD, Sigma Chemical Co., St Louis, MO, USA). Cytofluorimetric analysis was performed using a flow cytometer (FACSCalibur, BD, Bioscences, Franklin Lakes, NJ, USA).
Results: The automated procedure resulted in a median recovery of 89% (66-118; ±DS 11.01) nucleated cells and 86% (58-140; ±DS 17.8) CD34+ cells. The median viability, was of 60.66% (35-93; ±DS 14.7) within nucleated cells and of 88% (72-100; ±DS 9.03) within CD34+ subset. In addition, the recovery of hematopoietic progenitors (CFU-tot) was of 51% (14-76; ±DS 22.29). 33/34 patients engrafted with PMN count >0.5X10^9/L on two consecutive days sustained platelet counts >20X10^9/L without platelet transfusions observed after a median of 10 and 15 days after HCT respectively. Only one patient did not engraft due to complications associated to the bone marrow microenvironment. Subsequently the patient underwent allogeneic transplantation.
Conclusion: The DMSO washing procedure using Sepax S100 automated system do not affect the cell functionality assuring a satisfactory result in terms of nucleated cells, CD34+ cells recovery and viability.

P541

UCBT with low-dose ATG in adults

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Treatment by allogeneic hematopoetic stem cell transplantation (HSCT) is limited by availability of suitable human leucocyte antigen (HLA) matched adult donors. In Austria about 23% of all adult patients lack HLA-matched donors. Unrelated donor umbilical cord blood (UCB) has been increasingly used as an alternative HSC source for such patients.
We started UCBT at our department in 2001 and performed until now 27 UCBT´s in 21 patients: 13 AML, 5 Ph+ ALL, 1 CML-blast crisis, 1 CML chronic phase, 1 Multiple Myeloma (43; [21-60] yrs.; 13 male/8 female). 2 patients got a second UCBT due to primary non-engraftment and 4 additional patients were retransplanted after a preceding allo- or auto HSCT.
Myeloablative conditioning (n=11) consisted of Cyclophosphamide 60 mg/kg x 2 days, fractionated total body irradiation (fTBI) 1.200 cGy or VP16 60 mg/kg x 1 d/fTBI 1.200 cGy, whereas non-myeloablative conditioning (n=10) consisted of Cyclophosphamide 50 mg/kg x 1 d, Fludarabine 30 mg/m² x 6 d/fTBI 400 cGy. All patients received a Graft versus Host Disease (GvHD) prophylaxis: Cyclosporin for a minimum of 180 days after UCBT, Antithymocyte Globlulin (ATG Fresenius) 5 mg/kg x 4 d and Methotrexate 10 mg/m² day +1 and +3.
Nucleated cell dose infused for single UCBT was 3,52 (2-5,72) x 10^7/kg and 3,23 (2,74-4,21) x 10^7/kg body weight for double UCBT. CD34+ cell dose infused was 1,44 (0,21-5,6) x 10^5/kg for single and 1,19 (0,86-1,85) x 10^5/kg for double UCBT.
Concerning HLA match 75 % had a HLA mismatch 4/6, 25 % 5/6. In double UCBT no DRB1 mismatch, but A and/or B mismatch was allowed.

Time to neutrophil and platelet recovery was not different between single and double–unit recipients. Recovery of platelets ≥ $20x10^9$/L took place after 40 (0-56) days in single UCBT and after 40 (36-43) days in double UCBT. Neutrophil recovery > $0,5x10^9$/L appeared after 28 (15-42) days in single and after 29 (20-35) days in double UCBT´s.
The incidence of grade III-IV acute GvHD was remarkable low with 5% and grade I-II acute GvHD was 15%. Until now we could not observe extensive chronic GvHD.
Following the patients up to 120 months we observed an overall survival of 50% and a remarkable low incidence of GvHD in mainly heavily pretreated patients. For the future we plan increasingly to include patients with clear indication for UCBT in CR1 or CR2.

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Long-term storage of cord blood units: assessment of cell recovery and viability

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Cord blood (CB) banking supports the increasing use of this stem cell source for transplantation as both term and preterm umbilical CB contain a high number of early and committed hematopoietic progenitor cells.
Cryopreservation is a key point for the long term maintenance of viability and biological properties of CB cells. At present, the most used cryopreservation method is based on controlled cooling rate (-1°C/min).
The gained experience in bone marrow or peripheral blood showed that hematopoietic stem cells (HSC) can be effectively preserved for at least eleven years in nitrogen liquid, although successful reinfusions after longer time were seldom reported.
Data on CB cryopreservation refer to both relatively short and long term cryopreservation. In particular, Kobylka (1998)and Mugishima (1999) evaluated the flow cytometric and clonogenic characteristics after 12-15 years of cryopreservation. Considering the increasing time between CB collection and clinical use, appropriate validation of cryopreservation techniques is crucial for the feasibility and safety of this procedure.
We report here our experience in CB units stored beyond eleven years after collection.
Methods: 24 CB units stored in Florence CB bank in 1996 were thawed to assess the biological characteristics of cryopreserved stem cells. We evaluated the total nucleated cells (TNC), CD34+ cells and colony forming cells (CFC) recoveries and viability of TNC and CD34+ by annexinV/PI after thawing. At the time of the storage, CD34+ cells were evaluated with double platform assay (TNC Count and percentage of CD34+ cells on flow cytometer). After thawing, the CD34+ cells were evaluated with ISHAGE method (Single platform assay) as required by the actual quality standard practices.
Results: After a median storage time of 12,0 years (11,2-14,1), the average recoveries of TNC, CD34+ and CFC, and viability of TNC and CD34+ after CB thawing were reported in the table 1. We observed a full recovery of TNC, associated with a loss of CD34+ cells and CFC according to viability test results.
Discussion: 12 years post freezing, we observed an optimal recovery of TNC but an intermediated/low TNC and CD34+ cells viability associated with a loss of hematopoietic stem cells number, probably due to the different flow cytometric assays used before and after thawing. Standardization of processing and quality controls must be stably implemented in Cord Blood Banking activity.

	TNC Recovery (%)	CD34+ Recovery (Alive) (%)	CFC Recovery (%)	TNC Viability (%)	CD34+ Viability (%)
Average	108.6	49.3	73.1	51.1	42.9
Standard Deviation	17.2	18.0	40.0	6.7	14.5

P543

Predictivity of cord blood unit quality assessment on unrelated cord blood transplant outcome

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Background: Quality of Cord Blood Unit (CBU) significantly affects outcome of patients undergoing Cord Blood Transplant (CBT). CBU quality assessment (QA) is a multiple process with a final step performed by the transplant physicians at infusion. However, QA at the CBU pre-releasing point could represent a crucial check prior to the final CBU selection.
Objectives: The aims of this analysis consist in investigating the value of QA as prognostic factor of patient outcome after CB transplant. Materials and methods: our study includes 62 patients with a median age of 13.6 years (range 0.6-61) undergoing an unrelated CBT between September 2005 and September 2010 at the Transplant Centres of Rome Transplant Network (RTN). QA data collected from CB banks at pre-freezing and pre-releasing points and from Transplant Centers at infusion were sequentially compared: pre-freezing vs attached segment vs infusion vs pre-freezing. Comparisons between groups were made using Friedman test and the degree of correlation between the variables was evaluated by the Spearman correlation coefficient.
Results: Our analysis has shown a significant linear relationship between pre-freezing, pre-releasing and at infusion QA as NC and CD34+ cell dose ($p<0.001$). Infused CD34+ ($1.8x10^5$), as median value pro-kg of body weight, did not result significantly different from pre-freezing count ($2.1x10^5$) and pre-releasing ($2.2x10^5$) values. Infused CFU-GM ($1.82x10^4$/kg) did not result significantly different from pre-releasing QA ($1.64x10^4$/kg), while there is a significant difference between infused CFU-GM ($1.82x10^4$/kg) and pre-freezing ($3.35x10^4$/kg) data ($p=0.004$).
Conclusions: Missing pre-freezing CD34+ and CFU-GM values can be replaced by the pre-releasing results; moreover, pre-releasing CFU-GM value is highly related to the value at infusion so that it should be considered the reference value for the CBU choice.

P544

Efficacy of haplo-identical haematopoietic stem cells transplantation with different grafts in very high-risk acute leukaemias: unmanipulated versus partial manipulated

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Introduction: Haplo-HSCT is a treatment option for the approximately 70% of pts who don't have an HLA-matched donor. Principal causes limiting use of haplo-HSCT are severe acute graft-versus-host disease (aGVHD) following T-cell replete HSCT, graft failure, infectious complications and recurrent malignancy after T-cell depleted HSCT. Most of these complications depends on the cellular composition of the graft.
Aim: The purpose of study was to compare of haplo-HSCT efficacy with different graft: unmanipulated BM+PBSC and partial manipulated graft (selection of CD34+ cells from PB combined with unmanipulated BM) in patients with very high risk acute leukemias.
Methods: In the study 35pts were included, children and adolescents (1-21y.o)-29pts; adults-6pts (22-46y.o); ALL-20pts (CR2>-5pts; relapse-15pts); AML-15pts (CR2>-6pts; relapse-9pts). Conditioning regimens: MAC for 14pts, RIC for 21pts, prophylaxis of aGVHD: CsA-based-24pts, Tacro-based-11pts. Sources of the HSC: unmanipulated BM+PBSC-9pts, partial manipulated graft-26pts. Total quantity of CD34+ cells in partial manipulated graft was $10,4x10^6$/kg (2,4-30,7) and unmanipulated graft-$11,9x10^6$/kg (6,8-21,9).

Conclusions: Unmanipulated and partial manipulated haplo-HSCT is a feasible approach which promised high engraftment rates, reasonable TRM risks and stable OS in very high risk acute leukemias. It has become an important alternative option for patients who need urgent BMT in the absence of matched related or unrelated donors.

Results	Manipulated graft N=26	Unmanipulated graft N=9
100 days OS 68%	73%	55%
1 y. OS 37%	38%	33%
aGvHD (pts)	22	
O-I-II^0	87%	49,8%
III-IV^0	12%	49,2%
100 days TRM	19,2%	11,1%
Graft failure	7%	0%
Infections	11%	11,1%
1 y. TRM	53,8%	22,2%
Graft failure	19,2%	0%
Infections	34,6%	22,2%
Relapse	27%	55%

P545

Optimizing a programme of directed cord blood banking. The experience of the Pavia Cord Blood Bank

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Since it was established in 1996, our Cord Blood Bank (CBB) has collected and stored directed cord blood (DCB) for eligible residents of the Pavia region and for the patients of our hospital Paediatric BMT Unit, a centre of excellence where many nonresidents are treated. The presence of a designated recipient requires a well organized program connecting an integrated team of health professionals to prevent any setback that could damage the graft. As a FACT accredited institution, our CBB relies on the accuracy of training and continued competency of the personnel at the Collection and Processing Facility. Accurate arrangements are done with the family as documented by written documentation and regularly revised CBB procedures are shared with the referents of the collection centres who are specifically trained about. 24/24h receipt of CB units is guaranteed and the CBB staff is available for processing within 48 hours after collection, including weekends and holidays. Moreover the Pavia CBB benefits of an internal EFI accredited HLA Laboratory where experienced and dedicated staff provides HLA typing for both donors and recipients timely and effectively. Here we report on our program of DCB banking to evaluate its efficacy (see table 1). By December 2010, 179 DCBs were collected (168 for brothers/sisters and 11 for other siblings), whose characteristics are summarized in table 2. Eligibility to caesarean delivery was evaluted by the Obstretricians and not due to logistics. No adverse reaction on both newborn and mother was reported. All collections were banked except of two (1%) coming from two distant non fixed collection sites due to incorrect packaging and transportation. Prenatal HLA typing was assessed when amniocentesis was requested for diagnosis (haemoglobinophaties), while most of the units were typed after collection without delay in the transplant schedule. 80% (54/67) of matched CB units were transplanted, all except of 9 (17%) at the on-site Paediatric BMT Unit. Outcome data were available for 42/54 (78%) CBs, in particular no delivery/transport problems or adverse reaction at infusion was documented. 2 positive units were safely infused starting antibiotics according to the sensitivity spectrum and no sepsis occurred. In our hands, a strategy that centralizes the many expertises involved in DCB management was very effective and optimezes the resources by a hight transplantation rate and a non static and definitively costeffective inventory.

Table 1.

Collections facilities	
1. on-site (Pavia Obstetric Ward)	**120** (68%)
2. fixed (Pavia region)	**8** (4%)
3. non fixed (outside the Pavia region)	**51** (28%)
- neigbour	**12** (7%)
- distant	**39** (22%)
Recipient Diagnosis	
1. malignancies (leukaemia/lymphoma)	**71** (40%)
2. haemoglobinophaties	**63** (35%)
3.Fanconi A., Diamond-Blackfan A., neuroblastoma	**45** (25%)
Prenatal HLA typing	**41** (23%)
HLA identical DCB units	**67** (37%)
Transplanted DCB units	**54** (30%)
n= 179	

Table 2.

CB collections, n=179	
volume (ml)	**99** (25-199)
nucleated cell (NC) **content** ($x10^8$)	**956.39** (43.7-2287.5)
CD34+ cells number ($x10^4$)	**248.2** (8.9-1226.4)
viability (%)	**95.7** (54.7-99.9)
CFU-GM number ($x10^4$)	**398.7** (8.5-2112.5)
type of delivery (vaginal vs caesarean)	**77** (43%) **vs 102** (57%)
twin pregnancies	**8** (5%)
maternal age (years)	**33** (18-42)
donor gender (male vs female)	**97** (54%) **vs 82** (46%)
neonatal weight (g)	**3177** (1270-4720)
microbial contamination rate	**4** (2%)

Mean value and range in brackets (except for type of delivery, twin pregnancies and donor gender).

P546

Quality control test on cord blood units: a tool to check and validate the production process

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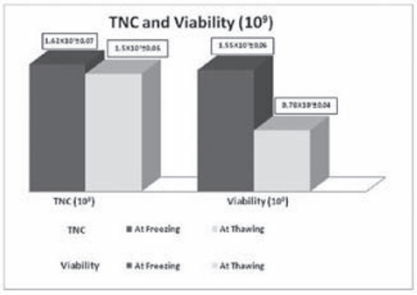
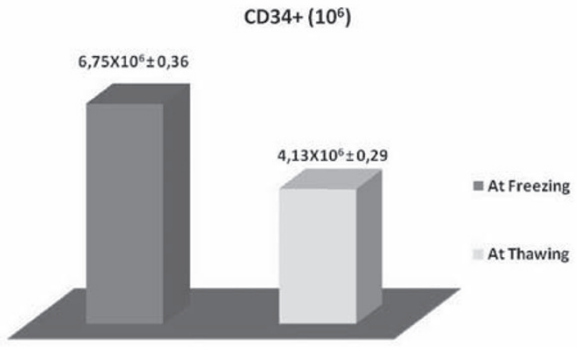
It is very important to make a prediction about the real amount of cellular content at thawing, since it coincides with infusion of the cellular product to the patient, when dealing with cryo-preserved cellular products for transplantation purpose, such as haematopoietic progenitor cells cord blood (HPC-CB) units.
The aim of this study is the standardization of an Internal Quality Control (IQC) on HPC-CB units in order to estimate the cord haemocomponents quality and to establish bank acceptability range parameters. IQC can provide reliable information about the efficiency of instruments used and allows to control the entire production process.
Quantitative and qualitative tests, on fresh product and on little amounts of product cryopreserved and stored with the correspondent unit, have been performed.
Especially, the following haematopoietic potential indicators have been investigated: total nuclear cells count (TNC), viability, CD34+ cell content, colony forming cell (CFU) content. In addition, sterility testing have been performed. From September to November 2010 n. 10 samples have been evaluated. An automatic cell counter (Symex XT 1800) has been used in order to determine TNC count. Viability has been determined using flow cytometric method (7AAD), CD34+ cells by flow cytometry with BD FACSCALIBUR and monoclonal antibodies (BECTON

DICKISON). In order to obtain CFUs, cell cultures have been incubated in culture medium (Methocult H4434 Stem Cell Technologies). The plates have been incubated for 14 days in a controlled environment (37 °C, 5% CO2, 85% humidity).
Our results showed high recoveries for TNCs (92%). The average cells viability between the samples was 51%. The recovery of vital CD34+ cells was 71% while the recovery of CFUs was 32%. All samples were negative to sterility control tests.
The tests performed have shown a very good yield rate for HPC-CB units. We discovered high recoveries for TNCs, CD34+ cells and viability. The growth of one colony at least in CFUs assures the presence of some stem cells in the cryopreserved HPC-CB unit. Despite the small number of samples, in our work we have identified some important indicators which can allow us to control the whole production process and to detect any possible non-conforming HPC-CB unit.





P547
Using other-related donors for allogeneic blood and marrow transplantation in Iran
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Objective: Sibling donors are generally the preferred donor source for stem cell transplantation. Unfortunately, by reducing the number of children in families, finding a matched-sibling donor is becoming more complicated. On the other hand, cord blood banks and HLA- registries have not been fully developed in so many other countries. It should be noted that in families where the parents are relatives, the chances of finding perfect donor will be increasing. So, in order to find a perfect match from such families a detailed family history, ideally in pedigree form, must be taken into account.
Methods: To extend the use of allogeneic HSCT to patients without an HLA-matched sibling donor, we investigated HSCT from other related donors. During 2008 to 2010, an early search for finding a related donor in the extended family was performed in all patients whose parents were relatives. High resolution typing for class I and II alleles was completed for recipient/donor pairs. By seeking in extended family, 62 patients with other related full matched donor transplanted. 39 patients were prepared for transplantation with antithymocyte globulin. Cyclosporine with or without methotrexate was used in graft-versus-host disease (GVHD) prophylaxis regimen.
Results: 62 patients (40 male, 22 female) with major thalassemia (n=17), fanconi anemia (n=9), ALL (n=8), AML (n=8), sever aplastic anemia (n=6), primary immunodeficiency (n=5), osteopetrosis (n=2), histocytic disorder (n=2) and hurler syndrome (n=2) were selected. Median age was 6 years (range: 4 months-54 years). The stem cell sources were bone marrow in 35 and peripheral blood in 27 patients. The donors were parents (n=41), uncle or aunt (n=12), grandfather or grandmother (n=5), cousin (n=2), great-aunt (n=1) and nephew (n=1) of these patients. All patients engrafted with neutrophils (>500/micro L) at a median of 14 days.22 out of 62 patients who had engrafted, developed Acute GVHD. Overall survival was 87.1%. TRM occurred in 3 patients (4.8%).
Conclusion: Considering the recent studies, outcomes of matched-other related transplantation are similar to those seen in transplantation with matched-sibling donors. So, in societies where consanguineous marriage is widely practiced, study of the extended family should be regarded as an integral part of finding the perfect donor process. Of course, it should be done before seeking cord blood banks and HLA-registries.

P548
Results of related and unrelated umbilical cord blood stem cell transplantation for 34 patients in Iran
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Introduction: Umbilical cord blood (UCB) transplantation is an available therapeutic option to a large variety of malignant and non-malignant disorders. By reducing the number of children in families, UCB can be considered as an alternative stem cell source for transplantation.
Patients and methods: In our center 34 patients have received 35 UCB transplantation from 1998 to 2010 (one patient was retransplanted). The male/female ratio was 24/10 with a median age of 5 years (range: 8 months-19 years). The most common disorders transplanted: thalassemia (n=9; 26.5%), acute lymphoblastic leukemia (n=7; 20.6%) and severe combined immunodeficiency (SCID) (n=4; 11.8%). The donor types for transplanted patients were 12 (34.3%) human leukocyte antigen (HLA) matched-identical siblings UCB and 23 (65.7%) HLA mismatched unrelated UCB (one locus mismatch: 4 patients, two locus mismatch: 18 patients). 4 patients received double-unit cord blood transplantation.
Results: The Median total pre-thawing nucleated cells infused were $4.1*10^7$/kg. The median time to Absolute Neutrophil Count $\geq 0.5*10^9$/L was +21(13-65 days). The median time to Absolute platelet count $\geq 20*10^9$/L was +29.5(16-62 days). Acute Graft versus Host Disease (GvHD) and Chronic GVHD occurred in 16(47%) and 2(5.9%) patients, respectively. The early mortality rate (up to 6 weeks) occurred in 7 patients (20.6%), 4 of 7 patients didn't have engraftment and died due to sepsis. During a median follow-up period of 3.5 months (9 days-11 years), 20 (58.8%) patients are still alive and 14(41.2%) are dead. The one-year overall survival (OS) is 61.7%.
Among 4 patients with double-unit UCB transplantation, 2 patients died due to relapse and sepsis on +81 and +92 days and 2 patients are still alive at the median follow-up of 14 months.
Conclusion: Nowadays, UCB transplantation is considered as a routine method in Iran. Moreover, seeking in Cord Blood Bank provides a potentially source for all of the allogeneic stem cell transplantations without a suitably human leukocyte antigen-matched related or unrelated donor.

P549

The determination of maternal micro-chimerism in cord blood units: is it a useful test in cord blood banking?

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During pregnancy, foetal hematopoietic cells migrate into maternal circulation and vice versa maternal nucleated cells can be detected in umbilical cord blood. This phenomenon, defined as maternal-foetal micro-chimerism, indicates the presence of bi-directional cell traffic between mother and foetus and probably stable long-term persistence of a small number of allogeneic cells in a genetically different organism. Maternal-foetal micro-chimerism may have a significant role in the field of umbilical cord blood transplantation (UCBT) and particularly in the development of GvHD and survival. Despite the relative phenotypic and functional immaturity of cord blood cells, acute and chronic GvHD may be encountered in UCBT recipients, although with a lower incidence and severity than other stem cell sources. Some studies showed that CB units can contain maternal cells that may be responsible of adverse sequelae. Therefore, the detection of contaminations by maternal cells may be of crucial interest.

Aim of this study was to determine retrospectively the maternal micro-chimerism in 22 UCB units (22 cords and 22 mothers) transplanted to HLA identical siblings. The recipients were children, aged from 1.5 to 11 years, affected by: thalassemia (9 children), Blackfan-Diamond anemia (5 children), ALL (4 children), neuroblastoma (2 children) and dyserythropoietic anemia (2 children). The median of total nucleated cells (TNC) infused was 4.5 x 10^7/kg (range: 2-14 x 10^7/kg). Maternal micro-chimerism was assessed by analysing selected short tandem repeats (STR) loci. A set of 5 paired primers, namely HumFGA, HumvWA, HumTH01, HumLPL, and HumCD4 was used. After screening, PCR analysis of CB units was carried out using at least two of the most informative STR loci for each CB unit/mother pair.

One out of the 22 CB units (4.5%) presented maternal micro-chimerism (10%), undetected by HLA typing standard molecular techniques. The recipient of this unit, affected by severe neuroblastoma, relapsed 9 months after UCBT and died.

We are unable to prove the real impact of maternal micro-chimerism in the survival post UCBT. However, it is reasonable to consider the chimeric unit like a mini-haploidentical transplant, due to the presence of maternal immuno-competent cells. The determination of maternal micro-chimerism, before transplant or at the time of banking, may be an additional test, qualifying the cord blood unit and justified by the relative frequency of this event.

P550

Reticulocyte maturation parameters as early predictors of haematopoietic engraftment afterumbilical cord blood allogeneic stem cell transplantation

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Introduction: We have previously shown that reticulocytes precede myeloid engraftment after Allogeneic Stem Cell Transplantation (Allo-STC) using bone marrow or peripheral blood. Umbilical cord blood (UCB) constitutes a hematopoietic stem cell source that is associated with a high percentage of graft failure and delayed myeloid engraftment. This prospective study aims to analyze reticulocyte maturation parameters as predictors of engraftment UCB Allo-SCT.

Methods: We included 37 consecutive patients undergoing UCB Allo-SCT from Jan-00 to Oct-10. Median age was 10 years (range: 1-34). Donor type was HLA identical sibling in 1 patient (2.7%), unrelated HLA-identical in 2 (5.4%) and HLA-mismatched donor in 34 (91.9%). Conditioning regimen was myeloablative in all patients (total body irradiation in 4 (10.8%) of them). All receptors received anti-thymocyte globulin as part of conditioning regimen. Median of infused CD34+ stem cells was 0.28x10^6/kg. Using daily drawn peripheral blood samples, we automatically measured in a Pentra 120 Retic: High Fluorescence Reticulocytes (RETH), Immature Reticulocyte Fraction (IRF), Mean Fluorescence Index (MFI) and Mean Reticulocyte Volume (MRV). We considered red-cell engraftment day to the first of three consecutive days to reach MFI ≥10, RETH ≥3%, IRF ≥10% and MRV ≥110 fl.

Results: A total of 35 (94.6%) receptors successfully reached myeloid engraftment while 2 (5.4%) patients presented graft failure; one of them received an autologous stem cell infusion rescue with normal hematopoietic restoration. Myeloid engraftment (>500 neutrophils) occurred on median-day +21 (range 15-48). Red cells engraftment based on reticulocyte parameters was detected earlier: RETH median was 15 days (range 9-43), IRF median was 16 days (range 9-45) and MFI median was 14 days (range 9-43). MRV median was 18.5 days (range 13-43). These differences reached statistical significance for MFI (P=0.012), IRF (P=0.017) and RETH (P=0.012) in Log Rank test. Fifteen patients did not reach MVR cut-off. The mean difference days between reticulocyte and myeloid engraftment were 6.63, 6.14 and 6.4 days, for MFI, IRF and RETH respectively.

Conclusions: After UCB Allo-SCT, red cells engraftment based on reticulocyte parameters occurs 6 days earlier than myeloid engraftment. This time-interval is very useful in order to take early clinical decisions, particularly when engraftment failure is suspected, to setup the time of a second stem cell infusion.

P551

Cord blood transplants: a single-centre experience

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Unrelated cord blood transplants (CBT) are used as alternative stem cell (SC) sources with increase frequency when no matched related or unrelated donor is found. In our centre, between Jun1989 and Sep 2010, 638 patients (pts) were submitted to 712 allogeneic SC transplants (alloSCT). In Oct1996 we performed the 1st CBT. In the last 10 years (y.) the median number of alloSCT performed each year is 48 (40-69); also in last 10 y. the median % of CBT is 8.7% (0-11.6%); in paediatric pts this median is 25% (0-80%).

Forty seven pts were submitted to CBT; in 1 (not further analysed) the SC source was the CB of his HLA identical twins brother, complemented with the bone marrow of 1 twin, due to the low number of cells. Forty-four CB were unrelated (43 non HLA identical), and 2 were HLA identical siblings. Two CB was used in 2 pts. Median age: 5 y. (5 months–49 y.); 21 male/25 female. Diagnoses: acute lymphoblastic leukaemia: 19, acute myeloid leukaemia: 9; myelodisplasic syndrome 5; bone marrow failure: 3; primary immunodeficiency; 3; osteopetrosis: 2; other: 5. Myeloablative conditioning (busulfan/cyclophosphamide based; ATG added in unrelated transplants): 40; reduced intensity conditioning (fludarabin/cyclophosphamide based): 6. Graft versus host disease prophylaxis: calmoudolin inhibitor+mofetil mycophenolate. Median number nucleated cells infused (x10^7/kg) and CD34+ cells infused (x10^5/kg): 5.8 (1.2-25.3) and 1.5 (0.1-8.8) respectively. Graft failure: 7 pts (2 deaths, 2 submitted to alloSCT with an alternative donor, in remission >2 and 4 y., 1 with autologous reconstitution in remission at 5 y., and 2 performed a salvage autologous graft, one died in relapse the other submitted 1 year later to an alloSCT of an unrelated donor and is in remission >8 y.). Graft versus host disease grade 2: 26, grade 3-4: 2. Overall survival at 5 y.: 48.5% (+8.7%); there was a trend of a better survival at 5 y. if infused nucleated cells> median (56.8+13.9% vs 38.7+11.0%; p: ns) and if CD34+ cells> median (68.0+12.5% vs 23.9+10.9%; p=0.01). Transplant related mortality at 3 y.: 23.3+6.5%. We found no difference

in TRM related to the number of cells infused. Median days to neutrophil recovery: 21 days; faster recovery if more than the median CD34+ cells infused (17 vs 28 days p=0.029). Median day platelets>20x10^9/L: 39.
Conclusions: despite slower rate of hematopoietic recovery, CB constitutes an alternative stem cell source that can be used when no matched donor is found.

P552
Haematopoietic progenitor cell collections using the Spectra Optia MNC protocol Version 5
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Objectives: We report a prospective, single-centre evaluation of the Spectra Optia® MNC device performance to harvest haematopoietic progenitor cells (HPC) from mobilized donors and patients.
The Spectra Optia MNC protocol uses centrifugation and a high-speed optical automated interface management (AIM) system to optimize the collection of a mononuclear cell (MNC) enriched layer.
During apheresis collection, MNC and platelets accumulate as a buffy coat at an interface layer above the red cell and granulocytes. The interface is optimally positioned for selective extraction by the AIM system. In a secondary step, platelets are separated from MNC and returned to the patient during the procedure, thus minimizing loss during the procedure.
Methods: Procedural data were collected from the Optia apheresis reporting system and laboratory analyses of pre-apheresis peripheral blood and harvested apheresis products. Data were analyzed for final product volume, MNC purity, residual red cell volume and CD34+ yield. Device performance was measured by CD34+ collection efficiency, expressing the total number of CD34+ cells collected as a proportion of the total number of CD34+ processed during apheresis. The same measure was applied to platelets to determine platelet loss to the product. Pre-apheresis CD34+/uL was plotted versus CD34 dose/kg and linear regression measured to determine predictability of yield.
Results: A total of 16 procedures were completed for 9 Autologous stem cell patients and 4 allogeneic stem cell donors, processing a median of 2.42 x total blood volumes in 267 minutes. Median pre-apheresis CD34+ counts was 63/uL (range 8–881).
Apheresis product and performance results are summarized below as median (range).
Conclusions: From these preliminary data we conclude that for a wide range of initial circulating CD34+ numbers, final platelet and CD34+ collection efficiency were good, MNC purity and red cell contamination were acceptable and final leucocyte counts and product volumes were within limits for overnight storage. Pre-apheresis CD34/uL was strongly correlated to final CD34 dose/kg allowing prediction of yield.

Final product volume (mL)	180mL (108 - 396)
Product WBC count	197 x 10^9/L (103 - 239)
MNC% Purity	83.5% (66% - 99%)
Residual RBC volume (mL)	3.0mL (1.6 - 12.3)
CD34 dose x 10^6/kg	4.68 x 10^6 (0.65 - 55.30)
CD34 collection efficiency%	50.4% (23.8% - 94.4%)
CD34/uL vs. CD34 dose/kg linear regression	r^2 = 0.9814
Platelet Collection efficiency%	14.1% (6.4% - 29.6%)

P553
Second allogeneic stem cell transplantation as treatment option for relapses, graft rejection or absence of engraftment after first allograft: single-centre experience
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Introduction: Allogeneic stem cell transplantation (SCT) is only potentially curative treatment option for different hematological malignancies. Despite efficacy, relapses are still possible and till now there is no standard approach neither for relapses, nor for other SCT failures. Current options for such cases are chemotherapy, donor lymphocyte infusion or second SCT.
Aim: We are reporting outcome of second SCT in our center.
Patients and method: From 1995 till 2009, 16 patients (pts) undergone second SCT for treatment of relapse (11 pts), graft rejection (2 pts) or absence of engraftment (3 pts) after first SCT. Median age in this cohort of pts was 22,5 (16-32) years, M/F ratio 10/6. Pts were suffering from various diseases: 2 AML, 7 ALL, 4 CML, 1 MDS, 2 AA. Median follow up is 30,5 (range 2-180) months.
Results: Disease relapse had occured at a median of 18,7 months after first allo SCT (range 6-72). 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 14 pts and bone marrow in 2 pts. Engraftment was observed in all pts with median neutrophil recovery after 16 (11-23) days. 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). Overall survival (OS) of all our pts is 37,5% with median follow up 60 (2-180) months.
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 need.

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The role of CD34+ cells, CFU and total nucleated cells in determining the target dose of stem cells prior to autologous stem cell transplantation
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Introduction: The most important factor for hematopoietic recovery (engraftment) after autologous stem cell transplantation (ASCT) is the dose of progenitor cells infused. Objective: retrospective study to assess the role of CD34+ cells, colony forming unite (CFU) and total nucleated cells in determining the target dose of stem cells prior ASCT.
Methods: Between 1994 and 2010, a total of 63 patients with acute myeloid leukemia (AML) in the Clinic of Hematology, Bratislava, received ASCT. 5 (8%) received bone marrow (BM), 53 (84%) received peripheral blood stem cells (PBSC) and 5 (8%) received BM + PBSC.
Results: patients transplanted with BM, PBSC and the combination of BM + PBSC attained a neutrophil count greater than 0.5 x 10^9/l a median of 24, 13 and 13 days post-transplant, respectively (P = 0.034). Similarly, the same subgroups of patients achieved a platelet count greater than 20 x 10^9/l a median of 66, 29 and 20 days after ASCT, respectively (P=0.076).

In patients who received BM SCT the median number of total nucleated cells, CD34+ cells and CFU infused were 1.4 x 10^8/kg (range 1.35–1.98), 2.34 x 10^6/kg (range 1.2–8.4) and 9.3 x 10^4/kg (range 2.4–26.6), respectively. Whereas in patients who received PB SCT the median number of total nucleated cells, CD34+ cells and CFU infused were 6.7 x 10^8/kg (range 1.9–38.9), 2.67 x 10^6/kg (range 0.52–32.50) and 93.4 x 10^4/kg (range 6.10–1463), respectively. Whereas in patients who received combination of BM and PB SCT the median number of total nucleated cells, CD34+ cells and CFU infused were 6.3 x 10^8/kg (range 3.5–11.4), 2x 10^6/kg (range 1.10–8.4) and 46.1 x 10^4/kg (range 4.8–137.5), respectively (Table 1). Nonparametric correlations were done using Spearman's test to determine the impact of the dose of nucleated cells, CD34+ cells and CFU on the engraftment. There was strong correlation between CFU and engraftment (Table 2).
Conclusion: In our study the engraftment after PBSCT was significantly shorter than after BMSCT, because the dose of progenitor cells infused in PBSCT was higher than in BMSCT. There was no statistical difference in the dose of CD34+ cells infused in patients with PBSCT and BMSCT, but the difference was in total nucleated cells and CFU. We also observed a strong correlation between CFU and engraftment, therefore CD34+ cells alone is a poor indicator for the target dose of progenitor cells to be infused and it must be combined with CFU.

Table 1. Dose of progenitor cells infused in 3 different types of autologous SCT

	BMSCT	PBSCT	PB+BM SCT	P
Total nucleated cells x 10^8/kg				**0.002**
Median	1.4	6.7	6.3	
Range	1.35 – 1.98	1.9 – 38.9	3.5 – 11.4	
$CD34^+$ cells x 10^6/kg				0.909
Median	2.34	2.67	2	
Range	1.2 – 8.4	0.52 – 32.50	1.10 – 8.4	
CFU x 10^4/kg				**0.001**
Median	9.3	93.4	46.1	
Range	2.4 – 26.6	6.10 – 1463	4.8 – 137.5	

Table 2. Correlation between total nucleated cells, $CD34^+$ cells, CFU and hematopoietic recovery

	ANC > 0.5 x 10^9/l	Platelets > 20 x 10^9/l
	P value	P value
Total nucleated cells	0.57	0.74
$CD34^+$ cells	**0.03**	0.079
CFU	**0.002**	**0.044**

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Umbilical cord blood stem cells for allogeneic transplantation
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Objectives: Umbilical cord blood (UCB) is good and safe source of hematopoietic stem cells (HSC) with high proliferative activity. UCB offers advantages comparing with other sources of hematopoietic cells.
Aims: to evaluate usefulness of UCB as a source of HSC for allogeneic transplantation in children.
Methods: 42 UCB are prepared according to strict criteria, Netcord standards and were used for allogeneic transplantation. 23 UCB HSC transplantation were made in 22 patients (3-ALL, 5-AML, 2-JMML, 4-MDS, 1-aplastic anemia, 8- mucopolysaccharidosis type I. Mean age was 5,8±0,42 (from 8 months to 20 years), 17 males and 5 females.
Results: 10 patients received 1 UCB sample, 3-2 UCB samples from the same donor, 2-2 UCB samples from different donors, 4-3 UCB samples (2 from the same donor and 3rd from another) and 1-4 UCB samples (2 from the same donor and 3 more from others). The choice of donors was made taking into account HLA compatibility and sample hemopoietic potential. In 3 cases donors were siblings, among these 2 HLA-identical sibling transplantation and 1 - transplantation one sample from sibling and another from non-sibling with 8/10 HLA-compatibility. Non relative allogeneic UCB HSC transplantation was used in 20 cases (HLA compatibility: 4/6-5 cases, 5/6-3, 8/10-6, 9/10-7, 10/10-3, 11/12-2). In 2 children BM HSC transplantation and co-transplantation of MSC from UCB were carried out. Mean transplant was 38 ml. Mean number of transplanted MNC 39x10^7, CD34+/CD45+cells 22.7x10^5 per kilogram of recipient weight. Primary engraftments succeed in 10 patients (mean time for neutrophil engraftment +21 day). Partial engraftment was made in 3 patients and in 7 cases we stated the failure of engraftment. We cannot evaluate the efficacy of procedure in 3 patients. Acute GVHD developed in 7 cases, chronic GVHD - in 1 patient. Transplantation-related mortality was 47% (9 deaths among 19 patients), in 4 patients death occurred due to severe accompanying diseases. Now 3 patients are in early post-transplantation period.
Conclusion: UCB is alternative source of HSC for allogeneic transplantation in the absence of HLA-compatible donors of BM or peripheral blood stem cells.

Paediatric issues

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Busulfan + fludarabine: an effective and low toxic conditioning regimen prior to HSCT in children with either malignant or non-malignant diseases
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Background: Busulfan as myeloablative agent is used in conditioning regimens prior to pediatric HSCT: mainly in combination with cyclophosphomide. We recently found a clear association between busulfan exposure and survival. However, toxicity leading to morbidity and associated mortality remains a limiting factor. Comparison studies in adults showed a favorable toxicity profile for fludarabine+busulfan (FludBu) compared to the conventional BuCy regimen. In paediatrics, limited data is available regarding this regimen. FludBu was recently introduced to replace the BuCy regimen in our center for myeloid malignancies and all non-malignant indications. We compared the outcomes with our BuCy historic controls.
Methods: Fludarabine 40 mg/m² was given in 1 hour prior to a 3 hour infusion of once daily busulfan (acc. to registered dosing regimen): for 4 days. The target area under the curve (AUC) for Bu was > 74 mg*h/L (cumm) in both groups. Busulfan dose targeting, based on therapeutic drug monitoring (TDM) was performed before the 2nd dose. Primary endpoints were event free survival (EFS) and overall survival (OS). Secondary endpoints were aGvHD, neutropenic period and the number of erythrocytes and thrombocytes transfusions. A risk factor analysis was performed using COX-regression models.
Results: 38 pts (28 unrelated-cord blood, 8 MSD and 2 MUD: 21 non-malignant and 17 malignant) were included in the FludBu group (median follow up median 187 days; range 22-769) and 44 in the BuCy group (975 days; range 6-1950). The groups were comparable regarding age, gender, indication for SCT and match-grade. More CB was used in the BuFlu group. EFS and OS in FludBu and BuCy was 73% vs 71% (NS) and 78% vs. 73% (NS), resp. No difference in aGvHD (≥ grade 2: 20 vs. 28%) was found. The period of neutropenia was median 11 in the FludBu group compared to 20.5 in the BuCy group (HR 0.38, p=0.05, CI95% 0.20-0.75). The median number of erythrocytes transfusion was 1 (range 1-13) in the FludBu group and 5 (0-22) in the BuCy group (p=0.04) and thrombocyte infusions 4 (range 0-33) vs 10 (range 2-44)(p=0,02). Less VOD was seen in BuFlu 3% vs. 18% in BuCy (p=0.02).

Conclusions: BuFlu with a total target AUC of >74 mg*h/l showed to be an effective and low toxic regimen. Interesting is the significantly shorter neutropenic period and lower number of transfusions needed in the FludBu group compared to BuCy. Although a small series, FludBu with TDM showed promising results.

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No differences in terms of toxicity and clinical outcome of intravenous busulfan versus oral busulfan as part of conditioning regimen for autologous and allogeneic stem cell transplantation in children: an analysis of the AIEOP-BMT Registry

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Objective: To compare toxicity, clinical outcome and compliance of iv busulfan (ivBU) versus oral busulfan (poBU) as part of conditioning regimen for autologous hematopoietic stem cell transplantation (autoHSCT) or allogeneic HSCT (alloHSCT) in pediatric patients (pts) with haematological disease or solid tumor.
Methods: Pts aged <18 year (yrs), treated with ivBU administered on accrual patient body weight dosage or poBU at dosage of 16 mg/kg from the AIEOP-BMT Registry between January 2004 (first pts receiving ivBU), and December 2009, were retrospectively analyzed to asses TRM incidence, grade II-IV hepatic toxicity risk by D+30 using Bearman criteria (HTR), and outcome. Pts were stratified for sex, age (infant vs adolescent), disease, disease status at HSCT (poor vs good risk), alloHSCT, autoHSCT, related (RD) or unrelated donor (UD), co administration of melphalan (L-PAM) or cyclophosphamide (CY), SC sources, I or II failure.
Results: 1007 pts, 157 receiving ivBU and 850 receiving poBU have been enrolled. For considering variables ivBU vs poBU: median age 6,64 vs 6,91 yrs, infant 26% vs 21%, adolescent 22% vs 21%; ALL 17% vs 6%, AML 23% vs 37%, NB 37% vs 19%, ES 19% vs 12%, NMD 4% vs 26% (p=.0001); poor risk 53% vs 40%, good risk 47% vs 60% (p=.009), autoHSCT 53% vs 34%, alloHSCT 47% vs 66% (p=.0001), RD 20% vs 37% (p=.0001), UD 28% vs 29%, L-PAM 69% vs 54% (p=.001), L-PAM and CY 45% vs 31%, Cord Blood 8% vs 11%, BM 41% vs 60%, PBSC 48% vs 27%, I failure 3% vs 5%, II failure 0 vs 3%.
After a median follow up of 345 (8-1850) days for ivBU and 445 (1-2182) days for poBU all pts have reached engraftment for PLT and PMN. For ivBU: overall 100 and 365 days cumulative incidence of TRM was 6.3 (2.3) and 7.3 (3.0) respectively; HTR was 19.5 %; 2 yrs overall survival 74.6 (4.8). For poBU: overall 100 and 365 days cumulative incidence of TRM was 5.1 (0.8) and 7.4 (1.0) respectively; HTR was 14.65%; 2 yrs overall survival 77.4 (1.7). In terms of TRM and HTR no statistical differences have been observed between ivBU and poBU for all the variables considered and particularly for pts affected by solid tumours who were treated with ivBU+L-PAM combination.
Discussion: In this large retrospective analysis TRM and incidence rate of HTR in pts treated with ivBu is low and not statistically different from poBU. No difference was observed in clinical outcome considering that a certain part of poBU patients had PK dose monitoring.

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Preliminary results of a phase II study using ch14.18/CHO antibody and interleukin 2 after haplo-identical stem cell transplantation in children with relapsed neuroblastoma

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Pediatric patients with relapsed neuroblastoma have a poor prognosis and additional therapeutic strategies are warranted. Current results of ongoing studies with haploidentical transplantation of T and B cell depleted stem cells and co-transfusion of donor NK cells show a 2 year-OS of 28% (n=25). Thus, transplantation itself seems to be not sufficient for most patients but may be a platform for further immunotherapies. In vitro stimulation with cytokines of donor derived patient NK cells posttranplant and/or use of chimeric antiGD2 antibodies resulted in excellent lysis of neuroblastoma cells. Thus, we started a phase I/II study to evaluate the feasibility and safety of anti GD2 administration in combination with low dose interleukin 2 after haploidentical SCT. Inclusion criteria: refractory disease or metastatic or nmyc+ relapse after previous autologous SCT. Primary endpoint is "success of treatment" defined as a patient receiving the full protocol treatment, still alive 180 days after treatment without progression and without unacceptable toxicity and acute GvHD Grade III or extensive chronic GvHD. Study design: 6 cycles of mAb CH14.18/CHO (20mg/m2 infusion for 5 days; in cycles 4-6, 1x10E6 units/m2 IL2 will be given additionally on days 6, 8 and 10). Clinical evaluations after 3 and 6 cycles (MIBG, catecholamines, MRI). Up to now, 8 patients received a total of 32 cycles. Side effects were: fever (26/32 cycles; median days > 38.5°C during mAb cycles: n=3 (0-5); highest frequency in 1st cycle, then decreasing); pain (32/32 cycles; median dosage of morphin i.v 22.5µg/kg/h); CRP elevation (25/32 cycles; median: 8.7 mg/dl) accommodation disturbance: 3 patients; loss of weight: 2 patients. No GvHD occurred. Current results: 6 patients were evaluated after 3 cycles. 4/6 patients responded to the treatment (mixed response n=1, PR improved, n=2, stable disease, n=1). 2 patients could be evaluated after 6 cycles up to now. One patient responded (VGPR improved), one patient progressed. In 5 patients, cytotoxity assays were performed. 3/5 patients showed a significant NK cell and complement mediated lysis of neuroblastoma cell lines during antibody infusions. Conclusions: haploidentical transplantation and subsequent antibody infusions should be further evaluated as a possible therapeutic option for relapsed neuroblastoma. First hints argue in favour of an existing anti-tumour effect. Our ongoing study will evaluate the feasibility and safety of this procedure.

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First clinical results with α-β+ T-cell depleted haplo-identical stem cells in children

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We have investigated the depletion of α-β+ T-lymphocytes via a biotinylated anti-α-β antibody followed by an anti-biotin antibody conjugated to magnetic microbeads in order to increase anti-tumor effects of haploidentical stem cell grafts. 5 pediatric patients with advanced and refractory leukemias (ALL, active disease n=3, graft failure n=1; AML, active dis, n=1) received

a haploidentical transplantation with such α-β+ T cell depleted grafts using the CliniMACS® system. Depletion of α-β+ T-cells was 4.5 log (range 3.8 – 5.0). The recovery of CD34+ stem cells, CD56+ NK cells and γ-delta+ T-cells was 72%, 76% and 80%, respectively. The median number of infused CD34+ stem cells, CD56+ NK cells and γ-delta+ T-lymphocytes was 11.9 x10^6/kg (range 7.5 -30 x 10^6/kg), 107 x 10^6/kg (range 35 -186 x10^6/kg) and 11.9 x10^6/kg (range 7.5 – 30.2 x 10^6/kg), respectively. The conditioning regimen comprised melphalan, thiotepa, fludarabin and OKT-3 from day -7 to day -1 in 4 patients and treosulphan, thiotepa, fludarabin and OKT-3 in 1 patient. No further post-transplant prophylaxis for GvHD was given. All patients showed a rapid engraftment with a median of 9 days to reach an ANC > 500 and 12 days (range 6 -21) to reach >20000 platelets. All patients had a complete donor engraftment and showed a rapid immune reconstitution with circulating donor-derived γ-delta T-cells first observed at day +3 followed by circulating donor-derived α-β+ T-cells first observed at around day +20. Median time to reach 100 CD3/µl was only 30 days. Despite the high number of infused γ-delta+ T-lymphocytes, only 1 patient experienced a grade 3 GvHD of the skin. 2 patients relapsed after transplantation, 3 patients are in remission for 0.46 (ALL, active dis.), 0.42 (AML, active dis.) and 0.26 (ALL, second trp) years up to now. In conclusion, the first preliminary experience of haploidentical transplantation with α-β T-cell depleted grafts showed a rapid and sustained engraftment, a rapid immune reconstitution and a low incidence of GvHD. A clinical trial in children and adults is underway to corroborate these promising preliminary findings.

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Role of IL15 stimulation of CD3/19 depleted transplants from haplo-identical donors in paediatric malignancies

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T and B cell depleted haploidentical grafts and a melphalan based intensity reduced regimen result in low toxicity and stable survival in patients with leukemias in CR. However, patients with active disease or second transplantation show unacceptable relapse rates. In an ongoing study, 36 pediatric patients with acute leukemias and advanced MDS received melphalan, fludarabine or clofarabine, thiotepa and OKT3. T and B cells were depleted by antiCD3/antiCD19 coated magnetic microbeads, whereas NK cells remained into the grafts.
Remission status was: CR1-3=18, NR=18, 18/36 already had received previous allogeneic transplantations. Relapse rates at 2 years were 20% (CR patients) and 73% (active disease or 2nd trp). Thus, we investigated options to reduce the risk of relapse by increasing antileukemic activity of donor NK cells in the grafts. Over night incubation with Interleukin 15 increased NK activity most effectively (specific lysis at E:T=20:1 against K562: 28% prior to and 71% after stimulation, n=10). After additional IL2 stimulation a 22 fold increase in thymidine uptake indicated proliferation of NK cells (n=5). No T cell proliferation was detectable. Based on these results, we started a pilot study with ex vivo IL15 stimulated grafts in 4 patients at very high risk (ALL, 2nd or 3rd relapse, active disease or CR (n=2); AML 1st relapse, active disease (n=2)). All patients received a backbone of unstimulated cells at day 0 to ensure engraftment. Additionally, parts of the grafts were incubated over night and infused at day +1 (medians: CD56=9.4x10^6/kg (range 3.7-24.4); CD34=1.5x10^6/kg; CD14=34x10^6/kg, CD3=0.01x10^6/kg). No side effects occurred. All patients engrafted within 12 days. 3 patients had acute GvDH grade 0-I, 1 patient had GvHD grade III. Recovery of NK cells was remarkably fast (526 CD56+/µl at day +14 versus 256 CD56+/µl in patients without IL15 stimulation). After additional administration of IL2 in vivo high NK activity (specific lysis>90% against K 562, E:T=20:1) was detectable in peripheral blood. Two patients are disease free (day +244 and 764), 2 patients died from relapse (day 56 and 64). Conclusions: ex vivo stimulation with IL15 strongly increases cytotoxic activity of NK cells in T and B cell depleted grafts and can counterbalance G-CSF mediated inhibitory effects. Those grafts were infused without any side effects and resulted in a fast recovery of functional donor NK cells. Further studies have to evaluate this approach.

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Once-daily intravenous busulfan-based conditioning for haematopoietic stem cell transplantation in pediatric patients: a large single-centre study

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Background: We analyzed the safety profile of i.v. high-dose busulfan-based conditioning in a large retrospective study in children. We use the weight-based nomogram without therapeutic drug monitoring.
Patients and methods: Between January 2006 and December 2010, 47 autologous and 139 allogeneic transplants were performed. In the autologous setting, median age was 4 years (range, 1-22) and 35 were male. Diagnosis was hematologic malignancy in 9 patients and solid tumour in 38 cases. High-dose busulfan was combined with melphalan in 32 cases, cyclophosphamide in 7 cases and thiotepa in the remainder 8 cases. Median CD34+ cells infused were 4,7 x 106/Kg (range, 1,8-58,7). In the allogeneic setting, the median age was 7 years (range, 1-19) with 92 male. There were 114 malignancies and 25 non-malignant diseases. Main diagnosis was ALL in 66 patients. Progenitor source was mainly peripheral blood. Graft manipulation was used in 97 patients. High-dose busulfan was associated with fludarabine and thiotepa in 106 cases, cyclophosphamide in 28 cases and fludarabine in 5 patients. Conditioning was myeloablative in 46 patients. Median CD34+ cells infused were 5, 3 x 10^6/Kg (range, 0, 1-69, 6).
Results: In autologous setting, neutrophil and platelet engraftment was achieved at a median time of 11 days (range, 8-16) and 12 days (range, 7-135), respectively. Severe mucositis (grade IV) was observed in 9 patients. Organ toxicity was only mild, with only 1 case of slight SOS. The only cause of death was relapsing disease in 11 patients. Disease-free survival was 69±8%, with a median follow up of 798 days (range, 48-1748). In allogeneic setting, neutrophil and platelet engraftment was achieved at a median time of 13 days (range, 7-40) and 14 days (range, 5-149), respectively. Severe mucositis was observed in 21 patients. Organ toxicity consisted on mild renal toxicity and gastrointestinal symptoms. There were not seizures or relevant neurologic toxicity. There were only 5 cases of moderated SOS. Severe acute and chronic GVHD incidence was 23±4% and 43±5%, respectively. The incidence of relapsing disease was 26±4%. Transplant-related mortality at day +100 was 16±3%. Disease-free survival was 53±5% for malignancies and 59±10% for non-malignant diseases. Median follow-up was 822 days (range, 19-1705).
Conclusions: Once-daily i.v. busulfan-based conditioning is a safety and effective regimen in pediatric patients undergoing hematopoietic stem cell transplantation.

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Impact of real-time sequence polymorphism (SP)-qPCR on post-transplant monitoring of haematopoietic chimerism in paediatric myelodysplastic syndrome

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Post-transplant monitoring of chimerism (chim.) allows surveillance of engraftment and detection of impending graft rejection or relapse (rlps) thus providing the basis for immunotherapy. However, impending rlps in advanced (adv.) MDS pts could not be detected in all pts using the gold-standard method STR-PCR. A possible reason is its quantifiable limit (1E-2). A more sensitive method might allow earlier and more frequent identification of significant mixed chim. (MC). SP-qPCR chim. monitors dynamics of autologous cells with high sensitivity (quantifiable limit 1E-4 to 1E-3) and reproducibility. (1) This principally offers the possibility of earlier and more frequent diagnosis of incipient rlps/graft rejection.

To substantiate this, we retrospectively performed SP-chim. In a multicenter study in 56 children with MDS after allo-SCT. This cohort was characterized by Bader in 05(2) and re-evaluated in this study. Previous STR-results were used for comparison. Pts: Female 19, male 37. Median age 11.1 (1.4-19.9). Dx: Refractory cytopenia 19, adv. MDS 37. Samples: 1076 (PB 875, BM 201). Median follow-up 2.7 years (0.3-6.2).

We showed previously that autologous cells >5E-3 (0.5%) correspond to significant MC in SP-qPCR.(3) The following risk groups were defined based on this threshold: High risk (HR) group: MC >0.5% in two consecutive samples, low risk (LR) group: all others.

Outcome: Low grade MDS (19 pts): HR group (2 pts): CR (1 pt received IT): 2 (100%), Rlps/TRM: 0 (0%). LR group (17 pts): CR: 15 (88%), Rlps: 0 (0%), TRM: 2 (12%). Adv. MDS (37 pts): HR group (10 pts): CR (5 pts received IT): 6 (60%), Rlps 3 (30%), TRM 1 (10%). LR group (27 pts): CR: 20 (74%), Rlps: 0 (0%), TRM: 7 (26%). Rlps risk was significantly higher in HR pts ($p<0.01$).

In 78% (7/9) of pts with MC, significant amounts of autologous cells were detected earlier by SP- compared to STR-PCR (median 43 days (18-740)). Rlps without previous significant MC did not occur in SP-chim. but in 1 case of STR-chim. Significant MC (qPCR) was found in 2 pts who remained in CR without intervention.

In adv. MDS, LR pts showed a trend towards higher 3-year EFS and significantly higher RFS estimates compared to HR pts (75%/100% vs 58%/65%, p=0.55/p=0.01).

In conclusion, in ped. MDS, qPCR allowed reliable monitoring of post-transplant state of chim. Substantial MC was detected earlier in 78% of cases compared to conventional PCR.

(1) Willasch, Lab Hematol 07, (2) Bader, BJH 05, (3) Willasch, BMT (suppl) 09, 10.

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Does unrelated peripheral blood remain an alternative graft source for children and adolescents with severe aplastic anaemia? Single centre study

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Matched sibling and unrelated donor bone marrow transplantations (MSD and MUDBMT) have been considered a therapy of choice in patients with severe aplastic anemia (SAA) due to a good overall survival (OS). In recent years PBSC instead of BM are increasingly used because of donor and/or centre preference. An earlier EBMT report identified higher incidence of chronic graft-versus-host disease (cGvHD) and mortality after transplantation of PBSC from HLA-matched siblings compared to BM in patients with SAA aged ≤20 years. A recent report by Eapen et al. confirmed a better OS for recipients of MUDBMT than MUDPBSCT (76 vs. 60%), however there was no difference in the rate of cGvHD in the pediatric cohort (23 vs. 26%).

As PBSC is a dominant source of stem cells for our patients with SAA undergoing MUD transplants, the outcome after MUDPBSCT (n=21) was compared with MSDBMT (n=13) performed in our institution in the years 1998-2010. Median follow up was 5 years. Compared with recipients of MSDBM, MUDPBSC recipients were in the same age (Me: 12.8 vs. 11.3 years, respectively) and received non-TBI containing conditioning regimes (FLU + CY or TREO/BU ± ATG/Campath). The interval from diagnosis to HSCT differed significantly between the groups (median time to HSCT was 3.5 months in MSDBM recipients and 16.7 months, in MUDPBSC recipients). The median day of ANC recovery was similar but platelet recovery was achieved later in recipients of MSDBM (16 and 33 days, respectively) than MUDPBSC (15 and 16 days, respectively). There were no significant differences in 5-year OS within studied groups (MSDBMT 0.85±0.10 vs. MUDPBSCT 0.59±0.11; p=0.163), as well as in 5-year event-free survival (0.61±0.14 vs. 0.59±0.11; p=0.725, respectively). The day-100 probability of severe grade III-IV aGvHD was similar after MUDPBSCT and MSDBMT (14.29%; N=3 vs. 7.69%; N=1; p=0.562, respectively). The probability of cGvHD was comparable within studied groups (MSDBMT 15.38%; N=2 vs. MUD-PBSCT 19.05%; N=4). One MSD BM and three MUD PBSC recipients developed extensive cGvHD.

These data suggest that:

1) outcomes after MUDPBSCT for SAA in children and adolescents are now comparable to outcomes observed in MSDBMT thanks to an excellent engraftment rate and fast and sustained hematologic recovery;
2) PBSC from unrelated donors remain an valuable alternative to well established MUDBMT;
3) The use of MUDPBSC does not increase the risk of cGvHD in pediatric patients with SAA (as shown by Eapen et al.)

P564

Unrelated bone marrow transplantation for patients with Fanconi anaemia: a single-centre experience in 28 patients transplanted with cyclophosphamide, fludarabine and ATG

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Introduction: Fanconi Anemia is a rare disease characterized by progressive pancytopenia, congenital abnormalities and a striking predisposition to cancer. Stem cell transplantation is the only curative treatment for the hematological complications related to this disease and the use of unrelated donors can be an option for those patients without a matched sibling donor.

Objective: Analyze the outcome of pts with FA submitted to an Unrelated Bone Marrow Transplantation (URD) using Cyclophosphamide, Fludarabine and ATG.

Material and methods: 221 pts with FA were transplanted in our institution from 1983 to 2011. In this study we included only pts submitted to an UBMT who were treated with the same preparatory and typed at least for locus A, B, C and DRB1. Number of pts: 28. Period: 02/2002 to 08/2010. Gender: 12F/16M.Age: 5-18ys (M: 10ys). 24pts received less than 25 previous blood transfusions. All pts had moderate or severe pancytopenia. No pt had overt MDS or AML and only 3/27pts had abnormal bone marrow cytogenetics. 4/28pts had mismatches at locus C. All

other pts were compatible at locus A,B,C (low resolution) and DRB1 (high resolution). Preparatory regimen: Cyclophosphamide 60 mg/kg + Fludarabine 125 mg/m^2 + rabbit ATG 4-5 mg/kg. GVHD prophylaxis: cyclosporine and methotrexate.
Results: 21/28 pts are alive between 90-2800 days after UBMT (M: 850d) with an overall survival of 75% at 3ys. Survival was excellent for pts < 10ys old (93% at 3 ys) and for pts compatible at locus A,B,C and DRB1 (85% at 3ys). 28/29 pts were evaluable for engraftment and 3pts died without platelet recovery. One pt had a primary graft failure (mismatch locus C) and was rescued after a second UBMT. Mucositis grade III (15pts) and IV (4pts) developed in 19 pts. Hemorrhagic cystitis: 6pts. Acute GVHD grade III-IV occurred in 4/26 evaluable pts while Extensive Chronic GVHD occurred in 6 / 21pts. 7pts died between 20 and 117 days (M: 50 days). Most pts died before 2007 (6pts) and the major causes of death were related to acute GVHD and infection.
Conclusion: UBMT for pts with FA has improved dramatically in the past few years especially because of a better selection of patients and donors. Acute and chronic GVHD are important complications and we need to decrease their incidence in this group of pts. Young children with bone marrow failures have an excellent prognosis.

P565
Overcoming female infertility after paediatric stem cell transplant: what can we offer?
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Background: Infertility is one of the most distressing late effects of stem cell transplant (SCT) in haematological diseases. Several new reproductive techniques are now available and ovarian cryopreservation (OCP) plays a leading role in paediatric population. In malignant diseases, in vitro maturation plus cryopreservation of aspirated oocytes and in vitro maturation of follicles within pieces of ovarian tissue are promising additional fertility-preserving methods.
Objective: To describe our programme for fertility preservation, including OCP, in a single centre cohort of SCT young girls with cancer.
Patients/Methods:
Descriptive study. Clinical records of children that underwent OCP in our SCT unit were retrospectively analyzed. Time period: October 2008- December 2010.
Explicit discussion with families and written consents were obtained, including assent of all transplanted minors.
Results: OCP was performed in our centre in 14 paediatric patients in the time period. Median age was 10, 2 years (3.5-18).
Patients´ characteristics that underwent SCT after OCP are provided in Table 1. Partial or entire laparoscopic ovariectomy was carried out without surgical complications. The tissue was successfully obtained at the same time of other surgical procedures: venous central line insertion (VCLI) bone marrow aspirate (BMA), lumbar puncture (LP). Preoperative imaging, histological examination of fresh tissue, immune- histochemistry, polymerase PCR or RT-PCR were used for excluding ovarian infiltration and confirming the presence of primordial follicles. We achieved in vitro maturation and cryopreservation of aspirated oocytes at the time of OCP in case 5 (5.1 years).
Comments: This technique is a new but increasingly successful clinical option and nowadays the only one that can be offered to young female patients.
In our experience OCP was safely and efficiently carried out. Close coordination between oncology, surgery and gynaecological teams was required to address practical and ethical issues in this vulnerable population.

Case	Dx	Age (yr)	SCT	Previous Chemotherapy	Time to next treatment (chemo/radiotherapy)	Surgery	Additional procedures at the same time
1	Metastatic Ewing sarcoma	13.5	Autologous	None	24 hours	Partial ovariectomy plus contralateral pexia	VCLI BMA
2	AML	15.6	PB/MRD	low gonadotoxic chemotherapy	1 week	Partial ovariectomy	BMA
3	B-ALL	14	autologous	low gonadotoxic chemotherapy	72 hours	Partial ovariectomy	BMA
4	AML	13.7	UCB/MUD	low gonadotoxic chemotherapy	72 hours	Partial ovariectomy	BMA
5	B-ALL	5.1	UCB/MUD	low gonadotoxic chemotherapy	96 hours	Entire ovariectomy	None
6	B-ALL	3.8	UCB/MUD	low gonadotoxic chemotherapy	72 hours	Entire ovariectomy	VCLI BMA LP
7	preB-ALL	3.5	autologous	low gonadotoxic chemotherapy	72 hours	Entire ovariectomy	None
8	B-ALL	7.5	autologous	low gonadotoxic chemotherapy	1 week	Entire ovariectomy	VCLI BMA
9	MDS	13.8	PB/MRD	low gonadotoxic chemotherapy	72 hours	Partial ovariectomy	VCLI BMA

P566
Poor immunogenicity of an adjuvanted pandemic influenza virus vaccine in immunodeficient children including allotransplant recipients
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Introduction: Immunocompromised patients including recipients of allogeneic haematopoietic stem cell transplantation (alloSCT) are at particular risk for severe infection with the pandemic 2009 H1N1 influenza virus (2009 H1N1) thus establishing the urgent need for efficient immuneprophylaxis. However, only very few data are available on the immunogenicity of 2009 H1N1 vaccines in this high-risk group, in particular in the pediatric setting.
Patients and methods: 2009 H1N1 virus-specific antibody concentrations were analysed via microneutralization test (MNT) in 45 pediatric patients (pts) suffering from haemato-oncologic diseases and/or immunodeficiency (alloSCT: n=12) who had been immunized with an adjuvanted 2009 H1N1 split-virus vaccine (Pandemrix). Rates of seroprotection (MNT titer ≥ 1:160) were calculated for the whole patient cohort (n=45). In a subgroup of 28 pts with available pre-vaccination serum samples seroconversion rate (% of vaccines with ≥4-fold titer increase and post-vaccination MNT titer ≥ 1:160) and geometric-mean-fold-increase of MNT titer were additionally analyzed.
Results: Seroprotective antibody levels after 2009 H1N1 vaccination were obtained in 49% (95%-CI: 35-63%) of all pts. When restricting analysis to 35 pts with T cell deficiency (i.e. those after alloSCT, undergoing chemotherapy, with inborn/acquired immunodeficiency and/or receiving immunosuppressive therapy) only 37% (24-54%) obtained seroprotective titers compared to 90% (57-100%) of immunocompentent children with haemato-oncologic diseases ($p<0.01$). Likewise, the seroconversion rate following 2009 H1N1 vaccination differed substantially between immunocompromised and immunocompetent children (17% vs 100%, $p<0.01$) and similar results were obtained for mean-fold-increase in MNT titer (1.7 vs. 22.6).
Conclusion: Our data demonstrate disappointingly poor immunogenicity of an adjuvanted 2009 H1N1 vaccine in immunocompromised children including alloSCT recipients leaving the majority of pediatric patients from this high-risk group unprotected. Thus, evaluation of alternative vaccination strategies is urgently needed. In this regard, we are currently running a prospective trial assessing the immunogenicity of the 2009 H1N1 vaccine component within the seasonal trivalent influenza virus vaccine in immunocompromised pediatric patients and preliminary data from this trial will be available in early 2011.
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P567

Superior outcome using cyclosporin A alone versus cyclosporin A plus methotrexate for post-transplant immunosuppression in children with acute leukaemia undergoing sibling haematopoietic stem cell transplantation

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Graft-versus-host disease (GVHD) is a major complication and one of the main causes of death after hematopoietic stem cell transplantation (HSCT). The GVHD prophylaxis may influence the incidence and severity of GVHD, relapse rate, and patient's survival. GVHD is associated with a graft-versus-leukemia effect, which is mediated by donor T lymphocytes and decreases the risk of relapse. Therefore, a reduced post-transplant immunosuppression might have a positive impact on patient's survival. The outcome of different immunosuppressive prophylaxis regimen for adult patients has been studied extensively, whereas the impact on children has yet to be determined. Therefore, we performed a study analyzing 62 children (median age, 11 years) with acute lymphoblastic leukemia (n=35) or acute myeloid leukemia (n=27) who underwent bone marrow (n=56) or peripheral blood stem cell (n=6) transplantation in a single center. All respective donors were HLA-identical siblings. All patients received an immunosuppressive prophylaxis consisting of cyclosporin A (CSA) plus methotrexate (MTX) (n=43) or CSA alone (n=19). All patients were given CSA at a total dose of 3 mg/kg/day. Patients in the CSA+MTX arm received MTX in addition at a dose of 10 mg/qm on days 1, 3, 6, and 11 and folinic acid at dose of 15 mg/qm, given 24 hours after MTX administration. Concerning the patient's gender, age, diagnosis, and state of remission prior to transplantation, the two groups did not differ significantly. Patients who received CSA alone as post-transplant immunosuppression had a significantly reduced cumulative incidence of relapse (5% versus 40%; p=0.002), a significantly increased 5-year event-free survival (84% versus 35%; p=0.001), and a significantly increased 5-year overall survival (84% versus 42%; p=0.004). Interestingly, the incidence of acute GVHD grade II-IV in patients in the CSA arm was equivalent to the CSA+MTX arm (26% versus 19%; p=0.440). In addition, we did not observe a significant difference in the cumulative incidence of chronic GVHD or transplant related mortality. In conclusion, CSA alone for post-transplant immunosuppression allows a stronger graft-versus-leukemia effect and reduces the relapse rate and the number of patients dying of leukemia effectively without increase of acute and chronic GVHD. Post-transplant immunosuppression consisting of CSA alone leads to a superior outcome and should be preferred in children with a HLA-identical sibling as donor.

P568

Conditioning with busulfan and fludarabine for allogeneic haematopoietic stem cell transplantation in children with acute myeloid leukaemia: a comparison to busulfan and cyclophosphamide

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Introduction: We evaluated the efficacy of fludarabine instead of cyclophosphamide combined with busulfan for hematopoietic stem cell transplantation (HSCT) in children with acute myeloid leukemia (AML).
Patients and methods: Outcomes of 54 children transplanted using busulfan and fludarabine (BF) were compared outcomes of 35 children transplanted using BuCy. All transplantations were performed between 2003 and 2009. From 2003 to 2004, BuCy was administered in all patients. From 2005 to 2007, both BuCy and BF were used but BF was usually administered in umbilical cord blood (UCB) transplantation. From 2008 to 2009, only BF was used in all patients. Fludarabine was administered at 40 mg/m^2/day for 5 days in bone marrow or peripheral blood transplantation and for 6 days in UCB transplantation.
Results: There were no significant differences in cytogenetic results and disease status prior to transplantation between two groups. However, the proportion of transplantation from alternative stem cell donors, such as UCB and haploidendical donor, was higher in BF group (48.1%) than in BuCy group (20.0%) (P=0.01). The rates of 100% donor chimerism in BuCy and BF group were 82.6% and 42.5% on day 28 (P=0.01), 100% and 72.2% on day 100 (P=0.01), 100% and 82.4% on day 180 (P=0.10), and 100% and 83.3% on day 365 (P=0.19) after HSCT, respectively. The median times to neutrophil and platelet recovery were 12 and 21 days in BuCy group, and 14 and 27 days in BF group, respectively. The incidence of grade 3-4 acute graft-versus-host disease (GVHD) were 6.1% in BuCy group and 9.4% in BF group, respectively (P=0.70). Rates of extensive chronic GVHD were 25.0% in BuCy group and 30.8% in BF group, respectively (P=0.60). VOD developed in 5 patients (14.3%) of BuCy group and 12 patients (22.2%) of BF group (P=0.35). Eleven patients (32.4%) of BuCy group and 7 patients (13.2%) of BF group showed hematuria within 30 days of HSCT (P=0.03). The 5-year probabilities of event-free survival and overall survival were 57.1% and 70.7% in BuCy group and 48.8% and 69.6% in BF group. Transplant-related mortality (TRM) within 100 days after HSCT occurred in 2 patients of BuCy group. One of them died due to high-dose cyclophosphamide induced cardiomyopathy. There was no early TRM in BF group.
Conclusion: Our results suggest that BF may substitute BuCy with an aim to decrease transplant-related toxicities without compromising its efficacy in children with AML.

P569

Failure of peripheral blood stem cell mobilization procedure in paediatric patients eligible to autologous transplant: a survey of the Italian Pediatric Hematology Oncology Association

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Background: High-dose chemotherapy with autologous stem cell rescue represents the standard treatment in pediatric tumours at high risk of relapse. In the last two decades, the collection of stem cells from peripheral blood (PBSC) by leukapheresis has largely replaced bone marrow harvest. We analyzed the incidence of the failures of mobilization procedure and morbidity related to the procedures.
Patients and methods: We collected prospectively from January 2009 to March 2010 the demographic and clinical data of 151 patients who underwent PBSC collection.
Results: They were 69 M and 82 F with a median age of 8 years, range 0.08-18 and a median weight of 25.5 kg, range 5-90. The underlying disease was CNS tumours in 48, neuroblastoma 37, Sarcoma/PNET 21, Hodgkin disease 16, acute leukemia 10, non-Hodgkin lymphoma 6, osteosarcoma 5, retinoblastoma 5, Wilms tumour 3. The status of disease at PBSC collection was CR and VGPR in 70 patients (46%), PR, stable disease or disease in 47 patients (31%), unknown for 34 patients (23%). Only 13 patients (7%) had received radiotherapy before PBSC. The G-CSF used was filgrastim in 107 patients, lenograstim in 39 patients, pegfilgrastim in 3 patients, unknown in 2 patients. The PBSC collection was successful in 121 of 151 patients

(80%) and a median of 1 leukapheresis was needed to collect the target of CD 34+ cell dose. Mobilizing chemotherapy was complicated by severe neutropenia in all 121 patients (median 5 days, range 1-45), mucositis in 21 of 151 patients (14%) lasting a median of 4 days, range 2-10, proven infection in 8 of 121 (7%) patients lasting a median of 4 days, range 1-12, or other toxicities in 5 patients (4%). Hospital admission for complications was needed in 30 of 121 patients (28%) for a median of 6 days, range 1-25. Nineteen of 30 (63%) patients who did not mobilized at the first time underwent a second mobilization procedure and 3 of 19 (16%) underwent also a third mobilization procedure while 12 of 30 (41%) patients required a bone marrow harvest.
Conclusions: In pediatric patients, current mobilizing chemotherapy resulted in a failure rate of 20%; moreover, a morbidity requiring hospital readmission for complications was observed in about 30% of cases.The availability of new mobilizing agents may potentially improve mobilization rate and decrease morbidity in pediatric patients.

P570
Pericardial Effusion after HSCT
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Introduction: Pericardial Effusion (PE) is a rare complication of Haematopoietic Stem Cell Transplantation (HSCT). Both alloreactivity and calcineurin inhibitor toxicity have a role in its development. The objective of our study was to identify incidence, risk factors, response to treatment and outcome of patients with PE after paediatric HSCT.
Patients and methods: We retrospectively included all patients transplanted at our centre between 2005 and 2010. Endpoints were the development of PE and overall survival. We analysed risk factors: patient factors (age, gender, underlying disease), HSCT details (donor source, HLA match, conditioning regimen) and complications (aGVHD, cGVHD, uremia and veno-occlusive disease).
Results: In 129 patients with a median age of 5.2 yr (0.16-21.2), HSCT indication was malignant disease in 71, 58 had a non malignant disease. Donor source was Unrelated Cord Blood in 59, Matched Unrelated Donor in 37 and Matched Related Donor in 33. 12 patients (9.3%) developed PE after a median of 71 (28-230) days after HSCT.
In univariable analysis non malignant indication (p=0.10) and young age (p=0.004) were significant risk factors for PE. In multivariate analysis only young age was significant. If expressed per year increase in age OR=0.41 (95%CI 0.47-0.93, p=0.017) Overall survival was 63 %, in the PE group 27 % of patients died, none from a PE related cause.
Although presenting symptoms were usually mild with respiratory distress and vomiting, echocardiography showed signs of tamponade in 8/12 patients. In all patients diuretics were started, and immune suppression was increased because of suspected alloimmunity. In eight patients immunesuppression was ineffective and only the discontinuance of calcineurin inhibitors resulted in the amelioration of PE. In patients with severe presentation a pericardiocentesis was performed directly (n= 4), in 4 other patients drainage was needed because of insufficient response to medical treatment. This procedure was safe and effective.
Conclusion: We report a PE incidence of 9.3%. Despite mild clinical symptoms, echocardiographic findings were often severe. Young age was the only significant risk factor. Not alloreactivity but calcineurin inhibitor toxicity seems to be the primary cause of PE. Pericardiocentesisis a safe and immediately effective procedure. PE did not influence overall survival. These data will be the basis for a treatment guideline, to be tested prospectively.

P571
Application of mesenchymal stem cells for paediatric steroid-resistant acute and chronic GvHD
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Background: Mesenchymal bone marrow-derived stroma cells (BM-MSC) are an emerging option for the treatment of steroid resistant graft-versus-host disease (GvHD). Although effectiveness and safety have been demonstrated in adults, there is few data on its use in pediatric patients. Here we report on a series of 5 children treated with BM-MSC at a single institution.
Methods: Steroid resistant acute GVHD was defined as incomplete response to prednisolone (48 mg/m²/d) after 14 days, refractory chronic GVHD as not achieving a partial response on prednisone (1 mg/kg BW) combined with a calcineurine inhibitor after 3 months. Patients received allogeneic hematopoietic stem cell transplantation (HCT) for mucopolysaccaridosis type 1 (MPS-I), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), sickle cell disease and severe combined immunodeficiency (SCID). MSC were generated under GMP conditions by an accredited manufacturer.
Results: Patients underwent HCT at a median age of 8.9 years (range 0.3-17.9). Donors were matched unrelated donors (3/5), a matched sibling (1/5) and a haploidentical family donor (1/5). GVHD involved the skin (4/5), the gut (4/5) and the liver (2/5). BM-MSCs were generated from the original donor in 3/5 cases and from a haploidentical sibling in 2/5 cases. A median dose of $4{,}5x10^6$ MSC/kg were given (range $0{,}1x10^6$ to $15x10^6$) in a median of 2 donations (range 1 to 7). Median time from diagnosis to MSC application were 80 days (range 60 o 237). The average dose was 2 x 10^6BM-MSC/kg per dose with a maximum of $4.0x10^6$/kg. Complete remission was observed in two and partial remission in three cases. Responses were observed within the first week after transfer. Liver-GvHD did not respond in both cases, although one patient received a total of 7 applications. MSC were tolerated well. No patient developed obvious adverse reactions with a median follow up of 36 months (range 12-55). The CML-patient died from relapse three years after HCT, the SCID-patient received a second HCT due to progressive liver-GvHD nine months after the first transplantation.
Conclusion: The few side effects after MSC application reported in adult studies are mirrored in our small pediatric cohort. Responses were seen in all children with skin and gut involvement. Liver disease did not respond to MSC treatment. These experiences warrant the effort of a prospective pediatric multicenter trial.

P572
Pseudoprogression after high-dose busulfan-thiotepa with autologous stem cell transplantation and radiation therapy in children with brain tumour: impact on survival
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Purpose: To determine the incidence, time course, predisposing factor and reversibility of neurotoxicity in children with brain tumors treated with high dose busulfan-thiotepa with autologous stem cell transplantation (ASCT) and radiation therapy in our institutional experience.
Patients and methods: We performed a retrospective analysis of prospectively collected data. Between May 1988 and May 2007, 110 patients, median age 3.6 years (range, 1 months-15.3 years) with brain tumors were treated with surgical intervention and conventional chemotherapy. All patients received one course of high-dose busulfan-thiotepa with stem cell rescue, followed or preceded by radiotherapy.

Results: Twenty-three patients (21%) developed neuroradiological abnormalities on follow-up imaging studies at a median time of 9.2 months (range, 5.6-17.3 months) after day 0 of ASCT. All MRI-lesions appeared in patients receiving radiotherapy after ASCT and were localized inside the 50-55 Gy isodoses. They disappeared in 14 of 23 patients with a median time of 8 months (range, 3-17 months). The presence of MRI-abnormalities was a favorable prognostic factor for overall survival on univariate analysis (hazard ratio: 0.12, 95% confidence interval [0.04, 0.33]), with a 5-year overall survival in patients with MRI-abnormalities was 84% (95% CI, 62-94), compared to 27% (95% CI, 19-37) in those without lesions. On multivariate analysis, the presence of MRI-abnormalities was an independent prognostic factor for overall survival.
Conclusion: MRI-detectable brain abnormalities are common early findings in children treated with high-dose busulfan-thiotepa followed by radiation therapy, and may mimic early tumor recurrence. They are correlated with a better outcome.

P573
Autologous stem cell transplantation for high-risk neuroblastoma: 20-year experience of a single paediatric institution in Brazil
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Introduction: The prognosis of high risk neuroblastoma (NB) is extremely poor. High-dose chemotherapy with autologous hematopoietic stem cell transplantation (AHSCT) has improved survival in the more recent years. We report here 76 patients that received AHSCT for high risk NB in our istitution.
Patients and methods: From 1989 to 2010, 76 patients underwent AHSCT for neuroblastoma in our institution. Median age was 5 years (39=M,37=F). Median follow-up was of 29 months, 36 patients were transplanted before 2001, and 40 patients after 2001. Seventy-one patients had stage IV-NB and 5 had stage III-NB with unfavorable biologic factors. Fifty-nine patients were in first complete remission or very good partial remission at the moment of transplant, 17 patients were in second or subsequent remission. The majority of patients (n=48) received peripheral blood stem cells (median CD34+ cells=2,6x10^6/Kg). Conditioning regimen consisted of carboplatin/etoposide/melphalan in 57 (75%) patients, melphalan only in 18 patients and bussulfan/melphalan in 1 patient. After 2001, a new protocol with AHSCT as consolidation treatment was started in our service and 27 of these reported patients were treated with this protocol.
Results: 71 patients engrafted in a median of 12 days. 2-year overall survival (OS) and 2-year progression-free survival (PFS) were, respectively, 43±7% e 38±6%. In a univariate analysis, among studied factors (age, diagnosis, disease stage, year of transplant), only the year of transplant had influence on both OS and PFS. 2y-OS and 2y-PFS were, respectively, 42±8% and 25±7% for patients transplanted before 2001 compared to 70±8% and 52±9% for patients transplanted after 2001 (p=0.02; p=0.028). Forty-one patients relapsed after AHSCT, in a median of 12 months. Four patients died of transplant toxicity and 36 patients died of disease progression.
Conclusion: We report here the 20 year experience of a single pediatric center in treating high-risk NB with AHSCT. Progression-free survival has improved over the years. This improvement may be related with better support measures, as infection prophylaxis and treatment, as well as the introduction of more intensive chemotherapy regimens. The inclusion of high-dose chemotherapy with AHSCT in the current treatment protocol may also have contributed for these results. A longer follow-up is although required to confirm these results in such high-risk diseases.

P574
Successful use of reduced-intensity conditioning for unrelated donor bone transplantation in children with dyskeratosis congenita associated bone marrow failure
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Dyskeratosis congenita (DC) is a rare inherited bone marrow failure syndrome with associated mucocutaneous/pulmonary abnormalities and cancer predisposition. Approximately 1/2 of cases have an identified mutation; for the remainder diagnosis is made by a constellation of family history & clinical findings in the setting of very short lymphocyte telomere length.Allogeneic bone marrow transplantation (BMT) is the only known curative therapy for the hematologic manifestations of DC but has been associated with increased transplant-related morbidity and mortality primarily due to hepatic, renal and pulmonary toxicity. Reduced intensity conditioning is thus attractive but there is no consensus on the most effective approach for these patients. Between 10/07 and 1/10 we treated 4 children at our institution with a reduced intensity regimen derived from a United States multi-institutional trial for aplastic anemia. The patients were 3-13 years of age at BMT. All had very short telomeres; in addition 1 had a genetic mutation (TERT), 1 a strong family history, 1 nail abnormalities and the remaining child had pervasive developmental delay. At the time of BMT all had tri-lineage cytopenias and hypocellular marrows with normal cytogenetics and no increased blasts. All received stem cells from a 9/10 (n=3, 1 A and 2 C mismatches) or 10/10 unrelated donor. Stem cell source was marrow in 3/4 and peripheral blood stem cells in one. The conditioning regimen was cyclophosphamide 150 mg/kg, fludarabine 120 mg/m^2, equine ATG 90 mg/kg and 200 cGy TBI. GVHD prophylaxis was cyclosporin and short course methotrexate. All patients successfully engrafted between D 17-32. One had renal insufficiency/ respiratory distress requiring a brief ICU stay without intubation. Another had a complicated course with renal insufficiency, cyclosporin induced seizure/ blindness, HHV-6 reactivation and respiratory insufficiency requring < 1 day of intubation. The other 2 had uneventful transplant courses. None had acute GVHD; 1/4 has chronic GVHD of the mouth and skin responding well to systemic steroids. At a median followup of 28 months (range 11-37) all are alive with performance status of 100%. Chimerism testing done between 4-10 months post-BMT revealed 100% donor chimerism for all. None have ongoing pulmonary, renal or hepatic toxicity. This reduced intensity approach appears to be associated with good engraftment, manageable peri-BMT complications and acceptable rates of GVHD and end-organ toxicity.

P575
Clinical outcome of patients with X-linked adrenoleucodystrophy who underwent allogeneic stem cell transplantation. Report from three Polish paediatric centres
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The aim of the report was to evaluate clinical outcome of patients with X-linked adrenoleucodystrophy (X-ALD) after allogeneic hematopoietic stem cell transplantation (HSCT) in Poland.
Patients: Polish Paediatric Haematopoietic Stem Cell Transplant Group registered 12 patients (pts) with X-ALD, who underwent allogeneic HSCT: 7 pts in Wroclaw, 3 in Poznan and 2 in Lublin. They underwent altogether 15 transplant procedures, because 3 patients had to be retransplanted due to late rejection/autologous recovery (median age at HSCT 104 months,

range 56-149 months). Primary conditioning regimen consisted of: Treosulfan (Treo) + Cyclophosphamide (Cy) (n=8), Treo-Fludarabine (Flu) -Cy (n=3) or Busulfan (Bu) + Cy (n=1) and Bu + Cy (n=1), ATG (n=1) or, Flu+ Thiotepa+ Melphalan (n=1) were used for second transplant procedures. ATG was used in all but one patients for GvHD/graft rejection prophylaxis. Nine patients underwent matched unrelated donor (MUD) and 3 pts matched sibling donor (MSD) transplantation in primary HSCT and 2 MUD and 1 MSD were used in subsequent grafts. The source of stem cells were: BM (n=6), PBSC (n=8) and BM+PBSC (n=1). Patients received median 7.35x10^6 CD34+cells/kg (range 1.22-23.54). Time from diagnosis to HSCT was median 11 months (range 2-36 months).
Results: Three of 12 patients (2 MUD, 1 MSD) rejected the graft after 3,12 and 15 months, respectively and were retransplanted. Seven of 12 patients remain alive in stable disease (eligible pts with Loes score < 8 pretransplant), 3 children remain alive in progressive disease (eligible pts with Loes score ≥ 8 pretransplant, time from HSCT to progression 1, 3 and 8 months, respectively), 2 died in progression of disease of a septic shock. Acute GvHD grade II (skin) developed in 6 patients and remained steroid-responsive. No extensive cGvHD was noted.
Conclusions: Allogeneic HSCT remains a potentially disease-stabilizing option in children with X-ALD irrespective of stem cell source and type of conditioning regimen.
The most important factor influencing the outcome is the neurological status pretransplant incl. Loes score. According to our observations, HSCT should be limited to children with Loes score < 8 pretransplant.

P576

Long-term defect of humoral immunity following administration of rituximab in children after allogeneic haematopoietic stem cell transplantation

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The monoclonal anti-CD20 antibody Rituximab (RTX) is increasingly used in allogeneic stem cell transplantation (SCT) to treat EBV DNA-emia or lymphoproliferative disorders (LPD), autoimmune anemia (AIHA) or other cytopenia and chronic graft-versus-host disease (cGvHD). However RTX administration leads to profound and long-lasting B-cell depletion and hence late recovery of humoral immunity.
For evaluation of recovery of humoral immunity in this study we report only those patients treated with RTX who are still alive at least 1 year after last dose of RTX (13-105; median 26 months). Between 2002 and 2009, 15 children received first RTX (dose 375mg/m^2) at median age of 2.9 years (range: 1.4-17.5 years) to treat EBV-LPD (4) or EBV DNA-emia (2), AIHA (9) or cGvHD (1) following allogeneic SCT. A total of 1–15 doses (median 6) of RTX were administered with first dose 2-30 (median 8) and last dose 5-107 (median 43) months after SCT. Two out of four patients treated for EBV-LPD did not respond and were subsequently successfully treated with high-dose chemotherapy. Per our standard practice, IVIG was given to children with pre-infusion (trough) IgG levels <4 g/L in absence of B-cells, or < 2 g/L if B-cells are present without frequent infections. At the time of last IVIG administration 12/15 patients were complete chimeras in non-separated peripheral blood (>90% leukocytes of donor origin).
Recovery of B lymphocytes, IgG, IgM and IgA was markedly delayed in 14/15 studied patients compared to standard population of transplanted children. Fourteen patients (93%) were therefore dependent on long-lasting administration of IVIG with 7 who still continue with IVIG 12.3-93.3 months after last dose of RTX. Absolute B lymphocyte count at 6 months after the last dose of RTX was below normal range (0.02-2.0 x10^9/L) in 4/13 patients (range 0–0.73; median 0.08) with improvement at 12 months after last RTX in 14/15 (range 0–1.06, median 0.31). Two patients temporarily treated with chemotherapy were not evaluated for 6 months B cell recovery. Despite IgG replacement 7/15 patients had frequent infections and/or chronic lung disease. Extensive cGVHD occurred in 4/15 patients.
Although several studies confirm the effectiveness of RTX for the above mentioned indications, long-term risks need to be studied before routine use of RTX for the above indications can be recommended.
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P577

Hyperbaric oxygen therapy for haemorrhagic cystitis post haematopoietic stem cell transplantation: a single-centre retrospective review

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Hemorrhagic cystitis (HC) is a frequent complication of hematopoietic stem cell transplantation (HSCT). Despite attempts to create an evidence-based algorithm for its treatment in children, the dearth of data available precludes the establishment of appropriate guidelines. We report the use of hyperbaric oxygen therapy (HBOT) in 13 children with HC after HSCT for malignant and nonmalignant diseases at our center from 2002-2010. The cohort's median age was 9.8 years (range 4-21), with 10 males and 3 females. Two patients (pts) received grafts from an HLA-matched sibling, the others from alternative sources (unrelated cord blood (2), matched unrelated donor (8), or haploidentical parent (1)). Conditioning included Melphalan (3 pts), Cyclophosphamide (5 pts), and total body irradiation (2 pts); 1 patient received both Melphalan and Cyclophosphamide. Symptoms began at a median of 33 days post HSCT (range 10-68). In 12/13 pts BK virus (BKV) was detected in urine by PCR; 9 of these pts had concurrent BK viremia. All BK positive pts received weekly Cidofovir. HC was Grade II in 1 pt, Grade III in 9 pts and Grade IV in 3 pts. Seven pts (54%) required intravenous narcotic therapy. The HBOT protocol included daily 90 minute treatments of 100% oxygen at 2 atmosphere. Pts received a median of 15 HBOT treatments (range 12-24). HBOT was initiated at a median of 9 days after bleeding began (range 1-37), and bleeding stopped by a median of 12 days from the initiation of HBOT (range 2-58) in the 11 pts whose hematuria eventually resolved (85%). One of the 2 pts who never cleared their hematuria remains with asymptomatic microhematuria 7 months post HSCT. One pt had subjective symptom improvement before starting HBOT; 1 pt had no symptom improvement, and in 2 cases subjective symptom improvement was not recorded separately from hematuria resolution. In 9 pts, symptoms began to improve at a median of 3 days from the initiation of HBOT (range 1-8). No pts experienced adverse effects from HBOT. Only 2 pts (15%) underwent invasive procedures, both requiring urinary catheter placement and cystostomies. Urinary catheterization was completely avoided in 11 patients. Our results suggest that administration of HBOT is a safe, effective therapy for children with HC. It alleviates symptoms, stops bleeding, and prevents invasive interventions. A multicenter randomized, controlled trial utilizing HBOT at presentation of HC would provide more concrete data as to its role in this setting.

P578

Changes in allogeneic haematopoietic stem cell transplantation in children during the last two decades

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Background: The protocols of allogeneic stem cell transplantation (ASCT) in children have been altered during the last two decades.
Aim: To compare outcome in pediatric patients receiving ASCT during 1992-2000 (P1) and 2001-2009 (P2).

Material and methods: We retrospectively analyzed 146 patients in P1 and 147 patients in P2. All patients were <18 years and a multivariate analysis was performed for outcomes; graft-versus-host disease (GvHD), transplant-related mortality (TRM), relapse, survival.
Results: The most significant protocol changes in P2 compared to the P1 were less myeloablative conditioning (MAC) containing total body irradiation (TBI), more reduced intensity conditioning (RIC) and altered GvHD- prophylaxis (p<0,001). Also in P2 we saw lower donor age, less treatment with granulocyte colony stimulating factor (G-CSF) and more peripheral blood (PB) and cord blood (CB) grafts replacing bone-marrow as stem-cell source (p<0,001). There was no difference in diagnoses or stage of disease between the groups.
TRM and relapse rates were not affected. Overall survival 3 years post ASCT were 58% in P1 as compared to 75% in P2 (p<0.01). The multivariate analysis showed that having a ASCT during P2 was associated with more acute GvHD (HR=1.76, p<0.01), which may be due to altered GvHD prophylaxis regimen. After correction for differences between the two groups, there was a trend for a better overall survival (HR=0.62, p<0.06) in P2.
Conclusion: During the last decade, changes in pediatric stem cell transplantation include more RIC-, PB stem cell- and CB transplants. We now see more acute GvHD and a trend for better overall survival.

P579

Reconditioning with total lymphoid irradiation and retransplantation from haplo-identical donors with CD3/CD19-depleted peripheral blood stem cells after graft rejection in paediatric patients

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Graft failure is a rare but life-threatening complication after hematopoetic stem cell transplantation. Treatment comprises immunoablative reconditioning regimens and a second stem cell donation as soon as possible to minimize the time of pancytopenia and its sequelae. We report a cohort of 14 pediatric patients with acute leukemias (lymphatic n=5, myeloic n=1), myelodysplastic syndrome (n=2), immunodeficiencies (n=3), paroxysmal nocturnal hemoglobinuria (n=1), and hemoglobinopathies (n=2) who experienced graft failure (nonengraftment n=1; rejection n=13) after busulphan or melphalan based myeloablative transplantation from mismatched related donors (n=12) or matched unrelated donors (n=2). All patients were retransplanted with CD3/CD19 depleted stem cells from a haploidentical donor. Median time from diagnosis of graft rejection to second transplantation was 19 days. Reconditioning regimens consisted of TLI (7Gray (Gy)) and fludarabine (120-150mg/m2) in combination with thiotepa (5-10mg/kg) and ATG/OKT3, one patient received cyclophosphamide instead of fludarabine. A median number of $15x10^6$/kg of body weight (bw) stem cells with $54x10^3$/kg residual T cells were infused. Mofetilmycophenolat was given as Graft vs. Host Disease (GvHD) prophylaxis, if residual T cells exceeded 25 000/kg bw. Sustained engraftment was achieved in 13 out of 14 patients (absolute neutrophil counts above 500/µl: 9 (9-11) days), one patient died before engraftment. Independence from platelet substitution was reached at a median time of 10 (7-22) days. Only 1 patient developed GvHD °III, 29% of all patients developed GvHD °I, 64% developed no GvHD at all. T cell recovery was delayed, however no lethal viral infection occurred. Severe organ toxicity was observed in 5 patients (bronchiolitis obliterans n=1, hemorrhagic cystitis n=2, leukencephalopathy n=2) and mucositis °3-4 in 13 patients. 10/14 patients are disease free (median follow up 1.45 (0.1-6.2) years); event free survival at 2 years was 67%. Causes of death were: multi organ failure (n=1), 3 patients with acute leukemias relapsed. Non of the patients rejected the second graft. Thus, transplantation of stem cells from haploidentical donors after reconditioning with TLI is a realistic option to rescue patients with graft failure within a short time span and for whom a second donation of the original donor is not available. The use of TLI (7Gy) before retransplantation from a different donor may help to avoid a second rejection.

P580

Effect of ATG on T-cell reconstitution post unrelated cord blood transplantation: A paediatric single-centre experience

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Early experience with unrelated cord blood transplantation used significant immunosuppression (ATG, cyclosporine with or without steroids) in an effort to reduce the risks of non-engraftment and acute graft vs host disease (aGVHD). However this combination has been associated with high rates of infection, especially viral infections. This has resulted in some groups reducing the amount of immunosuppression by removing the steroids. ATG continues to be used in varying doses especially in patients with non-malignant disorders. The high rates of viral infection post UCB is likely to be due to slow immune recovery, in particular T cell reconstitution.
There is little in the literature looking at the potential effect of ATG on T cell reconstitution post UCB transplant. In an effort to document this, we looked at all UCB transplants performed at our institution from June 1997–July 2010.
There were 52 UCB transplants performed for a variety of malignant and non-malignant conditions. Of these 23 patients had received ATG and 29 had not received ATG. The incidence of aGVHD was similar between the 2 groups. aGVHD contributed to the cause of death in 7 out of the 17 deaths in this cohort.
There were 35 patients who were alive at 3 months post-transplant. Information on lymphocyte reconstitution was available for 29 patients (13 in the ATG group and 16 in the non ATG group). CD3+, CD4+ and CD8+ counts were compared at 3, 6 and 12 months between those who did or did not receive ATG. CD3+ counts were higher at 3 and 6 months in the group who had not received ATG. CD4+counts were similar between the two groups at 3 and 6 months. CD 8+ counts were higher at both 3 and 6 months in the non ATG group. T cell reconstitution (CD3+, CD4+ and CD8+) was similar between the two groups at one year.
Although the numbers are small, these results raise the possibility of reducing the dose or removing ATG, especially in patients undergoing UCB transplants with malignant disorders in whom GVHD may be beneficial. Optimal ATG dosing has been investigated in adults receiving unrelated bone marrow or peripheral blood stem cell grafts. A randomised controlled trial addressing this question is warranted in paediatric patients undergoing UCB transplants.

P581

Outcome of patients with relapsed or progressive CNS germ cell tumours treated with high-dose chemotherapy followed by autologous stem cell rescue: results of Korean Society of Pediatric Neuro-Oncology-S 053 and 083 trial

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Introduction: The optimum therapy for relapsed or progressive (R/P) CNS germ cell tumors (GCT) remains to be established.

We investigated the efficacy of high dose chemotherapy followed by autologous stem cell rescue (HDCT/ASCR) in patients with R/P CNS GCT.
Methods: The patients with R/P CNS GCTs had been enrolled in the KSPNO–S053 and S083 protocols from 2004 to 2009. The treatment schema consisted of salvage chemotherapy ± radiation therapy ± surgery followed by single or double tandem HDCT /ASCR.
Results: Twenty-one patients from 6 hospitals had been enrolled. The median age at the diagnosis of R/P disease was 13.6 years (8.3~21.3 years). Only two patients had beyond second R/P disease. Fourteen patients (66.7%) had non-germinomatous germ cell tumors and 11 (52.4%) had more than M stage 2 at the time of R/P disease. The median interval of developing R/P disease after completion of initial therapy was 10 months (0~128 months). The 3-year overall survival (OS) and event-free survival (EFS) of all patients were 55.6% at a median 25 months and 45.5% at a median 19 months, respectively. Four patients did not undergo HDCT due to progression. One patient died of sepsis and 2 patients are under treatment waiting for a HDCT. Fourteen patients underwent HDCT and their 3-year EFS after first HDCT was 56.3% at a median follow-up 12 months. There was no significant difference in EFS between single HDCT (n=7) and double tandem HDCT (n=7) (57.1% and 53.6%, respectively, P=.887). However, the 3-year EFS of patients with complete remission (CR) (n=8) at first HDCT was 100%, but 0% in patients less than PR (n=6) (P<.001). There was no difference of EFS after HDCT according to M stage at R/P disease, tumor type, and number of R/P disease. No toxic death occurred during 21 HDCT procedures.
Conclusion: Even with limitation by small number and short follow-up period, the survival rates of current R/P CNS GCT study are encouraging. The tumor status prior to HDCT/ASCR was the most important factor for survival. Further study is necessary to develop the proper measures to achieve CR before HDCT, and to offer the best regimen for those patients with R/P CNS GCT.

P582

Retrospective analysis of stem cell transplantations for X-linked hyper-IgM syndrome

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X-linked hyper-IgM syndrome (XHIGM) is a primary immunodeficiency due to a mutation in CD40L gene. The patients are suffered to recurrent bacterial infections due to hypoγglobulinemia with normal to high IgM, and opportunistic infections due to the defect of costimulatory signals by CD40L to antigen presenting cells. Hematopoietic stem cell transplantation (HSCT) is recommended because the long term outcome is poor.
We performed 14 HSCTs for XHIGM patients. They experienced recurrent, severe bacterial infections as well as opportunistic infections such as P. jiroveci pneumonia (7 patients) and neutropenia (8 patients) before HSCT. Seven patients were trasplanted before 5 years old and seven patients were transplanted after 13 years old of age. Stem cell source was either bone marrow (11), umbilical cord blood (2) or peripheral blood stem cells (1) from HLA phenotypically identical sibling (4) or unrelated donor (10).
They received busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg) according to the guidelines of ESID/EBMT except for 3 patients with opportunistic infections (Cryptosporidium parvum or Tuberculosis) who received reduced intensity conditioning. Secondary graft failures were observed in 3 patients despite myeloablative conditioning. Eight patients suffred from infections during post transplant period and two patients died (day +63 and +89) and one patient had severe neurological complication due to encephalitis post HSCT. Severe acute GVHD (>grade 3) was observed in 2 patients. 4 patients had chronic GVHD but resolved after treatment. 12 patients were alive after 40 days to 11 years of HSCTs and 11 patients were free from IVIG substitutions. Although relatively high rate of graft failure should be overcome, we conclude that allogeneic HSCT from HLA matched related or unrelated donors including UCB is curative and feasible for XHIGM patients, if performed before significant infections and organ damage occur.

P583

Feasibility of planning peripheral stem cell harvest using regular chemotherapy schedules and granulocyte colony-stimulating factor as mobilization procedure in childhood cancer

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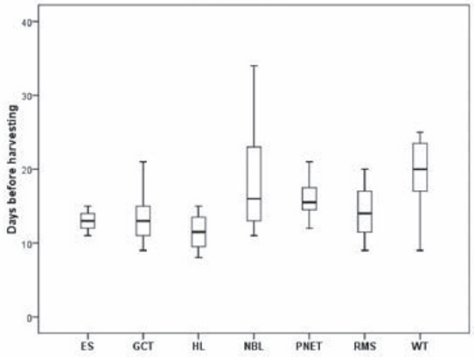
Background and objectives: Chemotherapy administered according currently best available treatment protocols, followed by granulocyte colony-stimulating factor (G-CSF) is the standard peripheral blood stem cell (PBSC) mobilization procedure in children with cancer. The optimal day to start collecting PBSCs is variable between the different chemotherapy regimens, dependent on type, stage, and the total cumulative dosages of administered chemotherapy before reaching remission status of the disease. Planning of the harvest procedure depends on real-time measurement of CD34+ cells in peripheral blood from the day of rising white blood cell count (WBC), which is time consuming and laborious and the optimal day for collecting PBSCs can be missed. Data on optimal planning of the PBSC harvest in children are not available at the moment. It would be a clinical and economical advantage if the optimal time point of PBSC mobilization following G-CSF supported chemotherapy in children could be predicted.
Methods: We retrospectively analysed mobilization parameters of 148 procedures in 90 pediatric cancer patients (neuroblastoma n=22, Ewing sarcoma n=22, primary cerebral neuroblastoma (PNET) n=12, relapsed Wilms tumor n=7, AML n=5, germ cell tumor n=5, relapsed Hodgkin lymphoma n=4, rhabdomyosarcoma n=3, other n=10). Patients were treated with chemotherapy regimens according to international protocols and subcutaneous G-CSF administration (10 microg/kg/day). To analyse the timing of all procedures we took the first day of mobilization chemotherapy as point of reference to calculate the days to time of harvesting. The start of apheresis was guided by WBC and CD34+ cell measurement in the peripheral blood.
Results. Median time to harvest of all 90 patients was 14 days. Timing of the harvest of peripheral stem cells in the different

Figure 1. Days before harvesting peripheral stem cells in children with different malignancies



types of tumors is described in figure 1. In 84 patients the target of 3-5 x 10^6 CD34+ cells/kg was reached. Four patients failed to mobilise a sufficient CD34+ stem cell yield (neuroblastoma n=2, peripheral nerve sheat tumor n=1, fibrosarcoma n=1); these patients underwent bone marrow aspiration.
Conclusion: In conclusion, a reasonable pattern which may guide the prediction of the moment of harvesting for each different type of tumour was found. This pattern was most specific for patients suffering from Ewing sarcoma and PNET.

P584
Haemorraghic cystitis in a cohort of paediatric transplants: incidence, treatment, outcome and risk factors
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Material and methods: We retrospectively analysed incidence, treatment and outcome of hemorrhagic cystitis (HC) in a cohort of 77 consecutive pediatric allogeneic SCTs between 2007 and 2009 in a single center. Risk factors for this complication were analysed.
Results: 77 transplants were performed in this time period. 7 transplants were excluded due to early death.
70 remaining transplants were analysed. They were done in 64 children (1 child received 3 sct's, 3 children were transplanted twice). Recipients ages ranged from 0-19 years (mean 7.1 yr). 19 received a HLA-identical related graft donor, 51 were unrelated transplants. Graft source was marrow in 33 cases, cord in 34 and PBSC in 3.
Conditioning regimens contained busulfan (bu) in 40 cases, Cyclophosphamide (cy) in 33, fludarabin (flu) in 24 and TBI/ VP-16 in 29 cases. Common chemo combinations were bu-cy and bu-flu. Cyclophosphamide was combined with hyperhydration and MESNA in all cases studied. GvH prophylaxis consisted of ciclosporin only in 45, in combination with methotrexate in 23 cases (other: 2).
Incidence of HC: there was no screening policy in our centre, urine was analysed in case of symptoms of dysuria or hematuria in the absence of bacteruria. 12 patients developed HC (17 %). In all but 2 cases it appeared after engraftment. The mean time period of first signs of HC was 14 days after engraftment (range 0-54 days). Severity was grade I in 1, II in 7, III in 4 cases. Aetiologic factors identified include BK virus (n=10) and 1 case of JC virus. All 12 affected children were treated. Treatment including forced hydration, spasmolytics and bladder irrigation was sufficient in 9 children. 3 children required cystoscopy, intravesical therapy and/or antiviral therapy. No child died due to this complication. Statistical analysis revealed only age over 12 years to be a risk factor for the development of HC.
Discussion: In our cohort, non-screened symptomatic HC occurred in 17 % of children. The literature indicates incidence rates of 10-70 %. Cyclophosphamide did not appear to lead to increased incidence of HC. The vast majority occurred after engraftment and seems related to poor immune function. All cases resolved, major therapy was only needed in a small proportion of patients.

P585
Health-related quality of life in paediatric patients after allogeneic stem cell transplantation – Development of a procedure-specific instrument
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Background: With increased survival after allogeneic stem cell transplantation(SCT) quality of life has emerged as an essential health outcome. The impact of transplant and chronic graft versus host disease (cGVHD) associated morbidity remains a major obstacle. The National Institutes of Health (NIH) chronic GVHD Consensus Project has recommended tools as independent measure to correlate with disease burden.
Methods: For the assessment of generic health-related quality of life (HRQL) we chose the PedsQL™ (Pediatric Quality of Life Inventory™) Generic Cores Scales, which has been used in a large number of healthy, acutely ill, chronically ill and severely ill children and adolescents from all over the world. To capture SCT- and specifically cGVHD-related problems, we developed scales by reviewing the literature, interviewing patients and parents as well as members of the health care team and by using the PedsQL™ measurement methods.
Results: The resulting tool consists of the HRQL domains pain and hurt, fatigue/sleeping problems/weakness, nausea, worry/anxiety about disease/treatment, nutritional problems, thinking/remembering, communication about disease/treatment and other complaints (pruritus, skin inflammation, problems with regard to mouth, eyes or breathing, loneliness). It was tested in a sample of paediatric patients referred to our SCT Outpatient Clinic between 100 and 360 days post SCT. These patients were followed up, if possible, at 3, 6, 9, and 12 months after the baseline survey. 24 % of patients were diagnosed with cGVHD. Both the PedsQL™ Generic Cores Scales as well as the disease-specific scales showed high internal consistency, with Cronbach α levels of ≥ 0,70 in patients' (n=52) and parents' (n=62) assessments. Most problems were seen in the HRQL domains of physical functioning and pain. The summary scores of the generic PedsQL™ and the disease-specific tool showed high correlations (r=0.89 in patients' and 0.81 in parents assessments). The tool can discriminate between patients with and without cGVHD.
Conclusion: The SCT-specific tool is practical for use and suitable across a broad age range (2-18 years). We suggest that up to 8 years the tool to be completed by parents, and after that age by both patients and parents.
In future work, we intend to test the tool in a larger population and examine whether additional items, e.g. with regard to physical symptoms, should be added in order to ensure high detectability of HRQL problems.

P586
Omitting methotrexate prophylaxis increases acute graft-versus-host disease in identical sibling transplantation in childhood leukaemia
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Background: In pediatric allogeneic stem cell transplantation (alloSCT) using HLA-identical donors, pharmacological graft-versus-host disease (GvHD) prophylaxis usually consists of ciclosporin A (CsA) with or without a short course of methotrexate (MTX). In accordance with I-BFM protocol recommendations for alloSCT in pediatric haematological malignancies, MTX has been omitted from our standard prophylaxis regime since 2007.
Objective: Evaluation of the influence of omitting MTX in GvHD prophylaxis on acute and chronic GvHD occurrence, neutrophil engraftment and immune reconstitution.
Patients and methods: A retrospective study was performed on a cohort of 38 consecutive and evaluable children, who underwent HLA-identical sibling alloSCT for acute lymphatic leukemia (n=24), acute myeloid leukemia (n=10) and chronic myeloid leukemia (n=4) in our transplant unit in the period 2000-2010. All patients received unmanipulated bone marrow grafts after myeloablative conditioning which was mostly busulfan or total body irradiation based. GvHD prophylaxis included CsA/MTX (group 1) or CsA only (group 2) in n=26 and n=12 patients, respectively.
Results: There were no differences in the numbers of infused nucleated cells between the two groups. Neutrophil engraftment occurred at day 25 and 20, respectively (p=0.1).
Acute GvHD grade II-IV occurred in 1/26 patients in group 1 versus 7/12 patients in group 2 (p=0.0001). Onset of acute

GvHD ≥ II within the first 30 days after alloSCT occurred in 0/26 versus 4/12 cases, respectively (p=0.001). Immune reconstitution was analysed at day 30, day 60 and day 90 post SCT. No significant differences were documented in numbers of CD3+, CD3+CD4+, CD3+CD8+ and CD3-CD16/56 cells when comparing group 1 and 2. More detailed analysis of early T cell reconstitution is ongoing. The number of children developing (severe) chronic GvHD was significantly increased in group 2 (p=0.027). With the limitation of the shorter follow-up of group 2 patients, no significant differences were found in relapse free survival between the two groups.
Conclusion: Omission of short course MTX as GvHD prophylaxis after HLA-identical sibling transplantation in pediatric leukemia patients is associated with a significant increase in (early) acute GvHD. The beneficial effect of acute GvHD on prevention of leukemia recurrence remains to be demonstrated.

P587

Gonadal function after haematopoietic stem cell transplantation: the single-centre experience

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The purpose of this study was to evaluate gonadal function in children and adolescents treated with hematopoietic stem cell transplantation (HSCT).
We conducted a retrospective study of 44 patients (26 boys) who underwent HSCT. 88% of patients were treated for hematological malignancies and 12% for other diagnosis. Autologous HSCT was done in 19 patients. Seven children were conditioned with total body irradiation. Gonadal functions were evaluated in children ≥ 9 years old. At the time of HSCT median age was 8.0 years. At the time of their last examination median age of boys was 15.6 and that of girls 16.5 years.
All boys entered puberty spontaneously and demonstrated age-appropriate levels of LH and testosterone. Fourteen (54.8%) pubertal boys had elevated concentrations of FSH. Boys with increased levels of FSH were significantly older at HSCT (12.8 vs 7.9; p=0.003). Median age at diagnosis of elevated FSH was 14.5 years. Six boys with elevated FSH had associated endocrine disorders: elevated HOMA-IR and subclinical hypothyroidism. Out of 18 girls, 16 (88.9%) had clinical and hormonal evidence of ovarian insufficiency (OI). The diagnosis of OI was established at median age of 12.3 years. Two of 16 girls recovered ovarian function after a period of 3 years. Among girls with OI, only one had spontaneous puberty and menarche. Because of delayed or arrested puberty, 9 girls required estrogen replacement therapy. Two girls were started on cyclic estrogen-progestin therapy. Three girls are still too young for replacement therapy. Twelve out of 16 girls with OI had one or more associated endocrine disorders: elevated HOMA-IR, subclinical hypothyroidism, thyroid carcinoma, GH deficiency and obesity.
Our results indicate that most boys undergoing HSCT can expect to enter and progress normally through puberty. On the other side, almost all girls treated with HSCT are in great risk of early OI. However, spontaneous recovery of ovarian function is possible.

P588

Result of haematopoietic stem cell transplantation in hemophagocytic lymphohistiocytosis with reduced-intensity conditioning regimen

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Objective: Haemophagocytosic lymphohistiocytosis (HLH) is a highly fatal disorder in children which represents the accelerated phase of some disorders such as chediak-Higashi syndrome, Griscelli syndrome and Familial erytrophagocytic lymphohistiocytosis (FEL). HLH can be devastating if appropriate treatment is not initiated. HSCT is the only curative treatment option for patients diagnosed with HLH. Since 2007, we have performed prospectively HLH transplantation with reduced-intensity conditioning (RIC) regimen which was due to their coexisting morbidity.
Methods: We prospectively analyzed the outcome of HSCT in 7 children with HLH from June 2007 to April 2010.During this study, one patient had a history of Chediak-Higashi syndrome, 3 patients had a history of FEL and 3 patients had a history of Griscelli syndrome before HSCT. 3 patients were in accelerated phase at the time of transplantation. The median age at diagnosis was 13.5 month (range: 1-54 month). The median age at transplantation was 22.8 month (range: 4-60 month). 6 patients were male. Six patients underwent full matched related donor transplantation (bone marrow in two, peripheral blood in four) and one patient was transplanted from 2 locus mismatch unrelated cord blood donor. All of the patients received fludarabine/melephalan/ATG-based reduced-intensity conditioning. The median numbers of MNC and CD34 injected were $6.35x10^8$/kg, $5.71x10^6$/kg, respectively (range: 2.31-11.74 x 10^6/kg). Cyclosporine and methylprednisolone were used as Graft-versus-host disease (GVHD) prophylaxis regimen.
Results: Engraftment occurred in patients. At the present time, 5 patients with median follow up of 22 months are still alive. Two patients died because of sepsis on days +16 and +165. They were in accelerated phase at the time of transplantation.2 patients who developed grade III-IV acute GVHD had a favorable response to therapy. Chronic GVHD occurred in one patient.
Conclusions: The results of this study shows that due to defect of the immune system which leads to co-morbidity such as severe infections in HLH patients, the use of RIC regimen with less toxicity instead of myeloablative conditioning regimens appear to be more reasonable. Early transplantation (in remission phase) and the use of reduced intensity conditioning regimen are suggested to improve patient satisfaction.

P589

Thrombomodulin early after allogeneic haematopoietic stem cell transplantation in children conditioned with FTBI versus busulphan-based regimen - preliminary results

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Background: Thrombomodulin (TM) acts as cofactor in thrombin-induced activation of protein C anticoagulant pathway. TM is expressed on endothelial cells, and released not only from these last cells, but also from platelets, monocytes, and neutrophiles.
Objective: Evaluate and compare TM serum concentrations in children conditioned for allo-HSCT with FTBI or Busulphan (BU) based regimens.

Patients and method: Total of 14 children, who underwent allo-HSCT between 2008-2009 were studied, including 8 (F/M=3/5; median age 8 years, range 1.5-16.5) conditioned with BU-based regimen (MSD/MUD/MMSD=5/2/1; BM/PBSC=5/3), and 6 (F/M=4/2; median age 10.5 years, range 6 15) conditioned with FTBI (MSD/MUD=3/3; BM/PBSC=3/3). As GvHD prophylaxis CsA was given, plus ATG and MTX in children undergoing MUD- or MMSD-HSCT. TM-concentration in serum was measured with ELISA (normal range: 2.73-4.79 ng/ml) before conditioning regimen, and then on day 0, +5, +11, +18, +25, +39.
Results: Before start of preparative regimen both in patients (pts) conditioned with FTBI and BU mean TM concentration was normal, i.e. 4±1,1 ng/ml and 4,3±1,7 ng/ml, respectively. Then in each of studied groups the TM concentration decreased significantly from day 0 (FTBI: 2,3±1,5 ng/ml; BU: 1,4±1,1 ng/ml) to day +11 (FTBI: 1,2±2 ng/ml; BU: 0,45±0,6 ng/ml), when the nadir was observed, and started to increase significantly from day +18 (FTBI: 3,1±2,8 ng/ml; BU: 3,7±3,6 ng/ml) up to its primary values on day +39 (FTBI: 6,4±4 ng/ml; BU: 4,2±2 ng/ml). Mean TM-concentration on day 0 was significantly lower in pts conditioned with BU-based regimen than in those conditioned with FTBI ($p<0.05$).
Conclusions: 1. Both in children conditioned for allo-HSCT with FTBI- and with BU-based preparative regimens significant decrease of TM serum concentration is observed within first 2 weeks after transplantation with subsequent return to primary TM concentrations within next 3 week. 2. On day 0 the decrease of TM serum concentration is significantly more pronounced in children conditioned with BU-based preparative regimens. 3. Significant decrease of TM serum concentration observed within first 2 weeks after allo-HSCT may contribute to impaired activation of protein C anticoagulant pathway, and thus to pathogenesis of early post-transplant coagulopathy and endothelial complications.
The study was supported by the Polish Ministry of Science and Higher Education (project N407 058737).

P590

Alloimmune reactions and resistant GvHD in paediatric patients following allogeneic stem cell transplantation – Mesenchymal stromal cells as a cell-based therapy

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Experimental evidence and clinical studies have demonstrated that human mesenchymal stromal cells (MSCs) have an immune modulatory function in the setting of allogeneic hematopoietic stem cell transplantation (SCT). The detailed mechanisms of action are not clarified yet. MSCs can be isolated from different tissues such as bone marrow of HLA-identical or non-identical donors. We used MSCs in four pediatric patients with steroid refractory GvHD (II-IV) with bloody diarrhea following SCT. All of them suffered also from severe hemorrhagic cystitis, in which BK-Virus was detected in urine. In addition to skin and gut GvHD two of our patients developed an alloimmune phenomenon in form of a severe haemolysis, acute pulmonary interstitial infiltrations, polyserositis with pleural and pericardial effusions and an overwhelming ascites. Both needed ventilation and intensive care measures. One of them received high dose steroids, Etanercept (TNF-α receptor blocker) and Toclizumab (IL-2 receptor blocker) without any positive effect.
MSC were cultured from bone marrow aspirates of HLA non-identical third-party donors. The average dose was 1 x 10^6 /kg, given intravenously. No acute adverse effects occurred during and after the MSC infusions. In all patients the MSCs had positive effect on GVHD, the hemorrhagic cystitis has ameliorated and transfusion requirements were reduced. After application of MSCs we observed also an improvement of the alloimmune reactions. The haemolysis was cleared and the pulmonary infiltrations, pleural and pericardial effusions regressed.
Three of four treated patients with MSCs are alive 3 months, 7 months and 3 years after SCT. One patient died of multi-organ failure due to an adenovirus sepsis.
In conclusion, MSCs demonstrated anti-inflammatory effects by ameliorating the consequences of the alloimmune reactions such as haemolysis, ployserositis, pulmonary infiltrations and ascites.

P591

Successful treatment of chronic granulomatous disease with myeloablative conditioning and allogeneic bone marrow transplantation – A single-centre experience

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Chronic granulomatous disease (CGD) is the defect of phagocytes resulting in recurrent infections and granuloma formation. Here we report the favourable outcome of allogeneic bone marrow transplantation after myeloablative conditioning in 6 high-risk CGD patients (1 female and 5 males, age 1–13 rears, mean 5) with severe disease related complications (sinusitis, overt pneumonia, skin, cervical and liver abscess, granulomatous colitis and gastroenteritis, sepsis, meningitis). Bone marrow donors were HLA–matched: related (2), unrelated (3), and HLA–mismatched unrelated (1). One patient was transplanted twice using the same sibling donor, because of graft rejection at 6 months after reduced intensity conditioning (RIC) transplant. The RIC consisted of fludarabine (150 mg/m²) and melphalan (140 mg/m²) whereas the myeloablative conditioning regimen consisted of busulphan (16-20 mg/kg) and cyclophosphamide (120–200 mg/kg). Patients transplanted from unrelated donors received rabbit thymoglobulin. Stem cell source was unmanipulated bone marrow containing: 5.2 (2.6–6.5) x 108 nucleated cells (NS), 3.8 (2.0–8.0) x 10^6 CD34+ cells and 45 (27–64) x 10^6 CD3+ cells per kg of recipient body weight. Graft versus host disease (GvHD) prophylaxis consisted of cyclosporine A (CsA) and, for unrelated donors, short course of methotrexate. CsA plasma concentration was kept between 150 and 250 ug/ml. CsA was continued until 3 to 6 months and then slowly tapered. Engraftment with full chimerism and functioning neutrophils were observed in all patients. Haemopoietic recovery occurred within a median time of 22 days (range: 20–23) to neutrophil count >500/μL and 20 days (range: 16–29) to platelets count >20000/mL. There was no episode of serious conditioning-related toxicity. Pre-existing infections and inflammatory granulomas resolved. One patient showed grade IV acute GvHD with the gut, liver, and skin involvement required therapy with prednisolone, mycophenolate mofetil, tacrolimus, oral budezonide, which finally resolved after anti TNF-a therapy and infusion of mesenchymal cells (0,3x10^6/kg) without continuing sequelae. With the follow-up of 2 to 32 (median 17) months, all patients are alive and well with full donor chimerism, normalized superoxide production, and T- and B-cell reconstitution.
Conclusion: We believe that it is desirable to perform bone marrow transplantation in young patients with proven diagnosis of CGD before the onset of life treating infections.

P592

Late engraftment in paediatric cord blood transplantation: lessons learned

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Background: There is a small risk of graft failure following stem cell transplantation, reported to be between 2-10% for adult patients. The data are limited in children and the largest paediatric studies relate to umbilical cord blood stem cells transplantation (UCB SCT). There are a number of factors, which increase the likelihood of late engraftment or graft failure. These include:

the use of mismatched donors, the use of cord blood stem cells, low CD 34 cell dose and T-cell depletion of the graft.
Objective: To define incidence, risk factors, treatment and clinical outcome of late engraftment/ graft failure in a single centre UCB SCT cohort.
Patients/Methods: Descriptive study. We retrospectively analyzed the clinical records of our patients undergoing UCB SCT. Time frame: June 1997-November 2010. All the patients had malignant hematological diseases. We selected patients who did not engraft by day +28 (recurrent pancytopenia with neutrophil counts <0.5 x 10^9/L and marrow aplasia) and registered demographic, clinical and biological variables.
Results: In this period, we performed 31 UCB SCT. The use of total body irradiation (TBI) and the GVHD prophylactic regimen varied upon used protocols in our Unit in each time frame.
We identified five late engraftment cases. All of them were unrelated transplants and received ATG. Patient characteristics at the time of transplantation are summarized in Table 1.
Patient number 2 was reconditioned and received a second UCB SCT due to autologous recovery (recipient 100% chimerism on day +32).
Comments:
- In our experience, morbi-mortality of patients who did not engraft before day +28 is high and mostly related to infections.
- Second SCT represents the therapeutic option with the best chance for long-term survival for patients with primary graft failure.

Table 1

1	11/9		2008	8/8					Yes			
2	7/1		2010	8/8					Yes			
3	3.2		1997	8/8					No			
4	5.2			8/8					No			
5	13.5		2008	8/8					Yes			

P593
Successful use of extracorporeal membrane oxygenation in a child with Hurler syndrome following allogeneic HSCT complicated by early RSV pneumonitis
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A 12-month-old boy with mucopolysaccharidosis type 1H (Hurler syndrome), pectus excavatum deformity and pre-existing chronic lung disease underwent peripheral blood stem cell transplant from a fully-matched (10/10) unrelated donor, seronegative for CMV. Cardiac structure/function was normal by echocardiography, and baseline lung function tests showed impaired lung function but oxygen independence. At time of transplant there was no evidence of infection. Conditioning therapy comprised IV busulfan, cyclophosphamide (200mgs/kg) and alemtuzumab (1mg/kg), according to our institutional protocol. Graft-versus-host-disease (GVHD) prophylaxis was with cyclosporine A and MMF. Conditioning was uneventful. At day+3 he developed fever and progressive respiratory failure due to respiratory syncytial virus (RSV) pneumonitis, requiring ventilation and subsequent extracorporeal membrane oxygenation (ECMO). This was complicated by pericardial tamponade requiring urgent sternotomy. After 22 days of ECMO he was decannulated and conventional ventilation continued. This was complicated by two spontaneous pneumothoraces requiring drainage. Despite these setbacks he was successfully weaned off ventilation and extubated on day+62. Throughout this time immune suppression was continued and no GVHD was seen. No other significant viral complications occurred. He was discharged, on home oxygen, 3 months post-transplant. He is now 6 months out and remains well with no significant transplant complications. He has full donor cell engraftment and is off immunosuppression. Oxygen therapy is being withdrawn.
ECMO is a means of respiratory and haemodynamic support for patients failing conventional ventilation. It is not commonly recommended in patients with irreversible airway disease, and its use in patients after HSCT is regarded as having very poor prognosis. In the largest reported series only 1 of 19 (5.3%) HSCT patients receiving ECMO survived to hospital discharge. Furthermore, amongst HSCT patients those with MPS1H, who often have pre-existing lung damage, are considered particularly poor candidates. Use of ECMO in this setting remains controversial. Our case demonstrates successful use of ECMO to support a child with MPS1H through RSV pneumonitis, with favourable outcome, survival beyond hospital discharge and no significant residual complications. We propose that ECMO can play a significant, life-saving role in management of post-HSCT respiratory failure in children with MPS1H.

P594
First case report of early cord blood transplantation in Farber disease: sustained engraftment does not prevent neurological symptoms
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Farber disease (FD) is a rare lysosomal storage disorder caused by acid ceramidase deficiency.
We report the case of a four month-old boy who presented typical painful subcutaneous nodules, joint deformations and contractures. Diagnosis of FD was confirmed by acid ceramidase deficiency in skin fibroblast culture. At nine months, he received an umbilical cord blood transplantation (UCBT) after a myeloablative conditioning regimen including busulfan and cyclophosphamide, and a graft versus host disease prophylaxis with cyclosporine, antithymocyte globulin and steroids. Before transplantation, he had no evidence of neurological impairment. Sustained full donor chimerism was obtained. Despite early and complete resolution of nodules and joint abnormalities after transplantation, hypotonia and delay in motor skill progressively appeared. Swallowing disorder began 18 months after transplant resulting in recurrent aspiration pneumonia, somewhat improved by percutaneous gastrostomy. Three years after transplant, his clinical status is almost stable with very little improvements of neuro-cognitive and motor skills.
In conclusion, UCBT is feasible with full engraftment and complete resolution of inflammatory symptoms. But this case confirms that early transplantation cannot prevent neurological manifestations of FD.

P595
Successful unrelated cord blood transplantation in a child with chronic eosinophilic leukaemia and constitutional trisomy 8 mosaicism
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Aim: Constitutional trisomy 8 mosaicism(CT8M) is known to have variable phenotypes, including multiple congenital anomalies, and the occurrence of a hematopoietic and solid neoplasia. Herein is reported the first case of chronic eosinophilic leukemia (CEL) in an 11 months(mon) old boy with CT8M successfully treated by tyrosine kinase inhibitor (TKI) and unrelated cord blood transplantation (CBT).
Case report: a 13 mon old boy admitted for evaluation of leukocytosis and hypereosinophilia diagnosed at the age of 11 mon. Blood count revealed hemoglobin 11gr/dl, leukocytes 50,000/µl, 90% of them eosinophils, and platelets 82,000/µl. Physical examination demonstrated hepato-splenomegaly and lymphadenopathy. Multiple lung infiltrates were demonstrated on lung CT. Bone marrow (BM) aspiration revealed multiple eosinophils, few blasts (<5%) and no monoclonality by immunophenotype analysis. BM cytogenetic analysis demonstrated abnormal karyotype, 47,XY+8 (trisomy 8). FISH analyses for

chromosome 16 inversion and t(9;22) translocation were negative. PCR analysis of JAK2, C-KIT and PDGFRA/FIP1L1 mutations- were negative. Skin biopsy revealed CT8M detected in 18/20 examined cells. As diagnosis of CEL was made, low dose of TKI, 50mg/day, in addition to steroid treatment, were initiated with prompt response. Later imatinib dose was raised to 100 mg/day due to a rise in leukocyte count. In view of the clinical course it was decided to proceed with BM transplantation. Since no matched sibling donor was found, unrelated donor search revealed matched cord blood- 6/6 with 3x10/5/kg CD34 cells and TNC-14.56x10/7/kg. Conditioning regimen included busulphan/cyclophosphamide/ATG. Graft versus host (GVH) prophylaxis included cyclosporine/prednisone. Leukocyte engraftment occurred on day +40. Last platelet transfusion was on day +70. Complications included: hemorrhagic cystitis, bacteremia (MRSA) and HHV-6 reactivation treated with Foscavir. Due to mixed chimerism cyclosporine was discontinued on day+95. He developed grade 2 skin GVHD and was treated with prednisone with good response. He is now 10 mon post transplantation without immunosuppression, no GVHD, disappearance of all disease manifestation including lung infiltrates, and full donor chimerism.
Conclusions: This is the first report of CEL in a child with CT8M. Clinical response to TKI points to the putative role of mutated TK in disease pathogenesis. Clinical course necessitated further treatment with successful CBT.

P596
Pure cell aplasia following allogeneic stem cell transplantation in children: a single-centre experience
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Background: Pure red cell aplasia (PRCA) is a well-known, although infrequent, haematological complication after allogeneic hematopoietic stem cell transplantation (HSCT). Paediatric information regarding this topic is scanty.
Aims: To study incidence, risk factors, treatment and clinical outcome of PRCA in our series.
Patients and methods: Descriptive and retrospective study. We have included every consecutively transplanted (alo-HSCT) patient in our Unit. Time period: February 1989- November 2010. Case definition: prolonged isolated red cell transfusion beyond day 60 after alo-HSCT and selective erythroblastopenia in full donor chimerism bone marrow, after exclusion of viral infections, alloantibodies and relapse.
Results: One hundred and twenty patients have been included (HLA- match related donor (MRD): 54, HLA-match unrelated donor MUD): 28, HLA-mismatch related donor: 7, umbilical cord blood (UCB): 31). All of them had malignant haematological diseases.
We identified four cases (3,3%) in the study period. Patients´ characteristics, types of transplants, PRCA treatment and outcome are provided in Table 1. They were all conditioned with radiotherapy based myeloablative regimes and none suffered acute, nor chronic GVHD.
Comments:
- PRCA incidence in our series is higher than expected according to adult reports.
- Only blood group O/A in recipient/donor pair was associated with the occurrence of PRCA in our series.
- Outcome was excellent (3/4 CR), even without any therapy.

Table 1

Case	Dx	Age (yr)	CR nº	HSCT nº	Source/Donor	CD 34*10⁶/kg	Blood group (Receptor/Donor)	Treatment	Outcome
1	preB-ALL (Ph+)	11	1	1	PB/MRD	6,05	O+/A-	EPO Rituximab	NR CR
2	T-ALL	5,1	1	1	PB/MRD	6,73	O-/A+	None	CR
3	Pre-B	8,7	1	1	PB/MRD	5,7	O+/A+	None	CR
4	B-ALL	13,8	2	2 (after autologous)	UCB/MUD	0,04	O+/A+	EPO Rituximab	NR NR

NR: No response. CR: Complete recovery

P597
Experience with marrow harvesting from paediatric donors at a single centre: safety and efficacy
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Objective: Bone marrow (BM) transplantation has become a standard treatment for various diseases, and children often serve as bone marrow donors for their siblings. This report presents the details of harvesting of BM through a total of 103 procedures performed in 100 pediatric donors aged 1–15 years.
Patients and methods: Between 1983 and 2010, 100 children (age, 1–15 years; median age, 9 years and weight, 10–100 kg; median weight, 27 kg) served as BM donors for their siblings. Three donors donated twice following primary graft failure or leukemia relapse. BMs were harvested from the posterior iliac crest under general anesthesia in all patients. Ninety eight of the 103 procedures required autologous red blood transfusion (75–600 ml), and reinfusion after red cell salvage was performed in 8 donors. All donors received intravenous antibiotics 3 times and oral antibiotics for 5 days after aspiration for prophylactically.
Results: The volume of BM aspirated varied from 153 ml to 950 ml (5–23.8 ml/kg donor body weight), and the transplanted cell content was 1.4–10.8 × 10^8/kg recipient body weight. The concentration of nucleated marrow cells in the BM harvests was 1.1–4.4 × 10^7/mL. With regard to cell yield, there was no significant difference between both harvests obtained from the 3 donors who donated twice. Although graft rejections were observed in 4 procedures performed in 3 patients (2 patients had metabolic disease and 1 patient had Fanconi anemia), the numbers of transplanted cells were sufficient (4.2–5.7 × 10^8/kg). The median Hb level in the donors before harvesting was 12.3 g/dL (range, 10.0–14.7 g/dL) and that after harvesting was 11.0 g/dL (range, 8.3–13.8 g/dL). The donors were hospitalized for 3–9 days (median duration, 4 days). Postoperative fever greater than 38.0°C was observed in 2 donors, and preoperative fever caused by pharyngitis was observed in 2 donors. All these episodes were resolved without complications. Asthmatic bronchitis developed in 1 donor after general anesthesia. The donor was treated with steroids. Most donors did not need analgesics for the pain at the harvest site, and 5 donors received oral analgesics; one of these 5 donors received intravenous pentazocine. All donors tolerated the procedures well, and no serious side effects occurred.
Conclusion: Our study indicates that children may safely donate BM for allogeneic transplantation and that substantial number of nucleated cells can be aspirated from them.

P598
Post-transplant immune reconstitution after allogeneic stem cell transplantation in paediatric patients with high-risk leukaemia
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Objectives: Immune recovery post allogeneic hematopoietic stem cell transplantation (HSCT) in young children is poorly defined. No guidelines exist 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 (4 ALL, 3 AML) with median age of 9 years (2-15yrs), 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). All patients had complete chimerism at 3,6, 12,24,36 months. Lymphocyte subpopulations CD3, CD4, CD8, NK, CD19, were available at 3,

6, 12, 24, 36 months and γ/delta T, CD45R0, CD45RA at 12 and 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,and H. influenza were tested at 12, 24, and 36 months post-transplant.
Results: CD3, CD8, CD19 normalized at 6 months, CD4 at 24 months, NK at 3 months, γ/delta cells at 12 months and CD45 RA, CD45 R0 at 36 months post HSCT. Mitogen proliferations were normal at 12 months and antigen proliferations between 12 and 36 months post HSCT. Antibody responses following vaccinations to all the antigens tested were normal at 12 months post HSCT; however there was a decrease over the follow up period especially in Pneumococcal antigens, necessitating revaccination.
Conclusions: This small retrospective study shows that for heavily treated and very high risk leukemia patients in the pediatric age, complete immune recovery is achieved 3 years post HSCT. This observation may be clinically important in providing guidelines regarding prophylaxis and the vaccination schedule in the long-term post transplant follow-up. Prospective studies in this group of patients are being planned to support the presented results.

P599
Successful treatment with low-dose gemtuzumab ozogamicin-combined chemotherapy followed by unrelated stem cell transplantation for children with refractory acute myeloid leukaemia
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The prognosis of children with acute myeloid leukemia (AML) who are refractory after relapse is dismal, even with allogeneic stem cell transplantation (SCT). To improve clinical outcomes of these patients, novel approaches should be applied to overcome the intrinsic resistance of leukemia cells against chemotherapy. Although gemtuzumab ozogamicin (GO), a monoclonal anti-CD33 antibody conjugated with calicheamicin, is a promising agent for AML patients who are resistant to conventional chemotherapy, little is known about its usefulness as cytoreductive therapy prior to SCT in children with refractory AML. Here, we report two cases of refractory AML successfully treated with low dose GO-combined chemotherapy followed by unrelated SCT with full intensity conditioning. Two AML patients of 7-year-old girl with AML1/MTG8 and 11-year-old girl with MLL/AF9 relapsed at 13 and 18 months from initial diagnosis. Because they were refractory to 2 courses of re-induction therapy, they received GO in combination with FLAG-IDA regimen as cytoreductive therapy prior to preconditioning for SCT; 15 mg/m² of fludarabine and 1 g/m² of cytarabine (day 1-4), 6 mg/m² of idarubicin (day 2-4), and 400 μg/m² of G-CSF (day 1-4). GO was administered at a dose of 3 mg/m² on day 5 of the treatment. To prevent veno-occlusive disease (VOD), danaparoid-based prophylaxis was initiated from the first day of the regimen. Both of two patients achieved complete remission on day 27 and 17 after the GO infusion, respectively, and they promptly received unrelated bone marrow or cord blood transplantation with the preconditioning consisted of 180 mg/m² of melphalan and 12 Gy of total body irradiation. The interval between GO and SCT was 40 and 27 days. In these patients, engraftment with full donor chimerism was obtained and no transplant related complications including VOD was seen. Despite a high-risk cohort of patients, they are alive and in remission at 12 and 13 months following SCT, respectively.
In contrast to the previous reports with concerns about GO related complications, we have shown that it is feasible to administer GO just prior to SCT with full intensity conditioning if the low dose of 3mg/m2 combined with danaparoid-based VOD prophylaxis is employed. In conclusion, low dose of GO-combined FLAG-IDA regimen followed by unrelated SCT with full intensity conditioning could be a safe and effective salvage therapy for children with refractory AML.

P600
Unrelated donor haematopoietic stem cell transplantation for children with neurodegenerative disorders
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Neurodegenerative disorders of childhood encompass heterogeneous diseases that result from specific genetic and biochemical defects showing progressive neurologic deterioration and dismal outcome. Globoid-cell leukodystrophy (GLD, Krabbe disease) is caused by a deficiency of the lysosomal enzyme galactocerebrosidase (GALC) leading to demyelination and death in early childhood. Adrenoleukodystrophy (ALD) is associated with the accumulation of saturated very long chain fatty acids (VLCFA) leading to progressive destruction of CNS white matter and adrenal cortex. With hematopoietic stem cell transplantation (HSCT), they can be effectively treated. Here, we report the experience of HSCT in the patients with neurodegenerative disorders.
Three children underwent unrelated HSCT. Two girls were diagnosed with GLD at 16 months, and 43 months of age, and one boy was diagnosed with ALD at 8 years of age. One patient received matched unrelated peripheral blood stem cell transplantation (PBSCT), and two patients received unrelated cord blood transplantation (UCBT). Conditioning regimen for PBSCT was busulfan, cyclophosphamide and rabbit-ATG. Busulfan, fludarabine, and cyclophosphamide were used for UCBT. The amount of transfused CD34 cells were $26x10^6$/kg, $1.5x10^5$/kg, and $2.6x10^5$/kg, respectively. Cyclosporine/methotrexate and cyclosporine/MMF were used for GVHD prophylaxis. All patients got hematopoietic reconstitution and showed 100%

[P600]

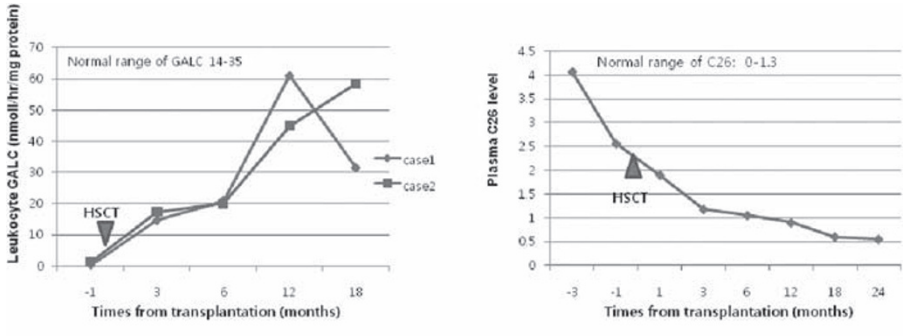

Figure 1. A) Leukocyte GALC activity before and after hematopoietic stem cell transplantation of the patients with GLD, (B) Plasm C26 levels of the patient with ALD decreased after hematopoietic stem cell transplantation.

donor chimerism. While two girls with GLD showed remarkable improvement in gross motor function and neurologic development after HSCT, the boy with ALD had rapid deterioration of neurologic symptoms before transplant, and he continued to get worse gradually until his neurologic deterioration stabilized after 1 year of transplant. Despite noticeable improvement of neurologic function was not observed, he seems none the worse after 4 years of HSCT.
We present the first trials of HSCT in Korea as the treatment for neurodegenerative disorders of childhood. They are curable diseases with HSCT, but early HSCT before severe neurologic deterioration is critical for a successful outcome.

P601

Can serum lactate dehydrogenase enzyme level be an early marker of myeloid engraftment in recipients of haematopoietic stem cell transplantation?

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Aims: In the present study, the relationship between myeloid engraftment time and serum LDH enzyme levels in patients, who underwent HSCT was studied.
Methods: The whole blood parameters and serum LDH, ALP, GGT, AST and ALT analyses of 25 patients, 24 of whom underwent allogeneic and 1 autologous HSCT were studied thrice weekly. The parameters on the onset of preparation regimen, the day, in which the product infusion was peformed, 5 days before myeloid engraftment, the day of myeloid engraftment and 5 days after engraftment were evaluated and statistically analysed.
Results: Allogeneic HSCT was performed on 9 patients because of Thalassemia Major, on 6 patients because of ALL, on 5 patients because of AML, on 2 patients because of FAA, on 1 patient because of JMML and on 1 patient because of HLH and autologous HSCT was performed on 1 patient because of AML. The AST, ALT and GGT levels of 3 cases were high before transplantation and remained high throughout the transplantation period. The other patients did not have any illness, which could cause high levels of LDH and G-CSF was not used in any patient. No statistically significant difference was present between the LDH levels on the onset of preparation regimen and on the infusion day (median 343 IU/L vs 374 IU/L, p>0,05). It was detected that the LDH levels on 5 days before myeloid engraftment started to increase with statistical significance (median 374 IU/L vs 433 IU/L, p<0.001), and that this increase continued on engraftment day and 5 days after engraftment and that there were no statistically significant difference between the levels on 5 days before engraftment, the day of engraftment and 5 days after engraftment (median 433 IU/L vs 456 IU/L vs 471 IU/L, p>0,05. It was also detected that the results were similar in the evaluation of cases with no elevation of transaminase levels and that LDH enzyme levels were not affected by high levels of transaminases.
Discussion: In the present study, it was detected that serum LDH levels begin to increase 5 days before myeloid engraftment and with the knowledge of intramedullary myelopoiesis lasts 5-7 days and postmitotic maturation period lasts 5-7 days, it can be suggested that this increase in LDH levels might be related to the increase of myelopoiesis in the bone marrow. In conclusion, the sequential follow up of serum LDH levels in patients undergoing HSCT and the detection of the period of increase in enzyme levels can herald subsequent engraftment.

P602

Successful haplo-identical stem cell transplantation in a patient with chronic granulomatous disease

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Chronic granulomatous disease (CGD) is a primary immunodeficiency characterized by the deficient generation of reactive oxygen metabolites by phagocytic cells. As a decisive prophylaxis and antibiotic therapy for bacterial and fungal infections usually allows the control of infections for many years, the indication for Hematopoietic Stem Cell Transplantation (HSCT) has been confined to patients for whom HLA-identical donors can be identified.
We report on a patient who was diagnosed with X-linked CGD at birth (positive family history). Despite antibiotic (Cotrimoxazole) and antifungal (Itraconazole) prophylaxis since early infancy he suffered from recurrent lymphadenitis, chonic inflammatory bowel disease, multiple bacterial pneumonias and at the age of 10 years developed pulmonary aspergillosis complicated by fungal osteomyelitis of his 4th rib. This complication could neither be controlled by repetitive surgery nor systemic antibiotic and antifungal therapy for a period of more than 4 months. As no HLA-identical donor could be identified he received a T cell depleted haploidentical transplant (donor: father; CD34+:1x10e7/kg; CD3+: 0,8x10e4/kg) after conditioning with Busulfan (i.v. 17,6mg/kg), Fludarabine (150 mg/m^2), Thiotepa (10 mg/m^2) and Campath (1 mg/kg). Except for a prolonged period of 12 months with low T cell counts the transplant course and follow up of more than 1 year was completely uneventful.
Summary: In selected patients with CGD and invasive infections not manageable with conservative or surgical measures, HLA-haploidentical HSCT is a therapeutic option. As patients with CGD have an isolated defect of phagocytic cells and normal lymphocyte function they are most probably less threatened by viral disease after T cell depleted HSCT compared to patients with malignancies or primary T cell deficiencies.

P603

Safety and tolerability of donortype red blood cell transfusion before allogeneic stem cell transplantation in children with major ABO mismatch

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Background: Major ABO mismatch in allogeneic bone marrow transplant (BMT) can cause clinical problems like severe transfusional reactions, acute haemolysis, delay of red blood cell (RBC) engraftment or manifestation of pure red cell aplasia, related to circulating isoagglutinin titers. Even there is no increased incidence of graft failure or slower engraftment of neutrophils and platelets ABO mismatch leads to an increase of transplant related mortility.
To reduce circulating isoagglutinin titers pre transplant plasma exchange during conditioning period can be performed. This procedure can be wearing for the patient and rebound effects are observed. RBC depletion can cause a loss of CD34+ stem cells in bone marrow. A further strategy to reduce circulating isoagglutinin titers is in vivo immunoadsorption due to donortype RBC transfusion pre transplant.
Methods: From 2007-2010 13 children (median age 7 years, range 0.9-18) received an ABO mismatched RBC transfusion (2-3 ml KG BW) pre transplant under antihistaminic and steroid cover. Reaction to donortype RBC and graft transfusion, haemolysis paramters and the trend of isoagglutinin titers were observed.

Results: In this retrospective study we present the experience of our transplant center. During RBC transfusion no severe complications are observed. 10 children presented no reactions, 1 child had a minimal oxygen demand and 1 child showed a mild hypertension and 1 child a moderate hypertension during transfusion. Haemolysis parameters were increased in 3 patients (LDH max. 1400 U/l and bilirubin max. 1.8 mg/dl). All patients showed a significant reduction of isoagglutinin titers post donortype RBC transfusion and no reactions to ABO mismatched graft infusion.
Conclusion: Donortype RBC transfusion is a safe and tolerable procedure to reduce the isoagglutinin titers prior to allogeneic ABO mismatched bone marrow transplantation in children. Further studies with more patients are needed to evaluate more detailed information on RBC transfusion post transplant and the RBC engraftment to establish this procedure as a standard therapy in ABO mismatched bone marrow transplantations.

P604
Moderate exercise increases natural killer cell cytotoxicity after allogeneic paediatric haematopoietic stem cell transplantation
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Objectives: The purpose of this study was to investigate the effect of moderate physical exercise on Natural Killer (NK) cell phenotype and cytotoxicity during the early period after allogeneic T cell depleted graft stem cell transplantation in pediatric patients.
Methods: 8 patients were randomly assigned, with 4 assigned to an exercise group and 4 to a control group. A Bachelor of Science in Physical Activity and Sport designed moderate physical exercise program (strength and endurance). Each exercise session took about 60 minutes and was scheduled 3 times a week during 10 weeks. Physical training program consisted on mixed supervised (at intrahospitalary gym) and home-based exercise program using Wii device, Wii Fit and/or Wii Sports software. NK immune phenotype by multiparametic flow cytometry and cytotoxic activity against K562 cell line by real time fluoerescence were measured before and after physical intervention in both groups.
Results: Patients in the exercise group have a more mature NK cell phenotype and an increase on NK cell cytotoxicity (Figure 1).
Conclusion: Moderate physical exercise should be a NK cell stimulus early after allogeneic T cell depleted graft stem cell transplantation in pediatric patients. These findings suggest that moderate exercise could be a beneficial adjuvant post transplant tool in paediatric allogenic stem cell transplantation.

P605
Allogeneic bone marrow transplantation in severe Glanzmann's thrombasthenia complicated by antiplatelet alloimmunization
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Glanzmann's thrombasthenia (GT) is an autosomal recessive disorder characterized by a lack of platelet aggregation due to the absence of platelet glycoprotein IIb and IIIa. Stem cell transplantation is curative, but it is only indicated in selected patients with a severe clinical phenotype or who develop anti-platelet antibodies.
We report the case of a 13-month-old boy, presenting with very severe type 1 Glanzmann's thrombasthenia, successfully treated with an HLA-identical sibling bone marrow transplant (BMT) complicated by antiplatelet alloimmunization.

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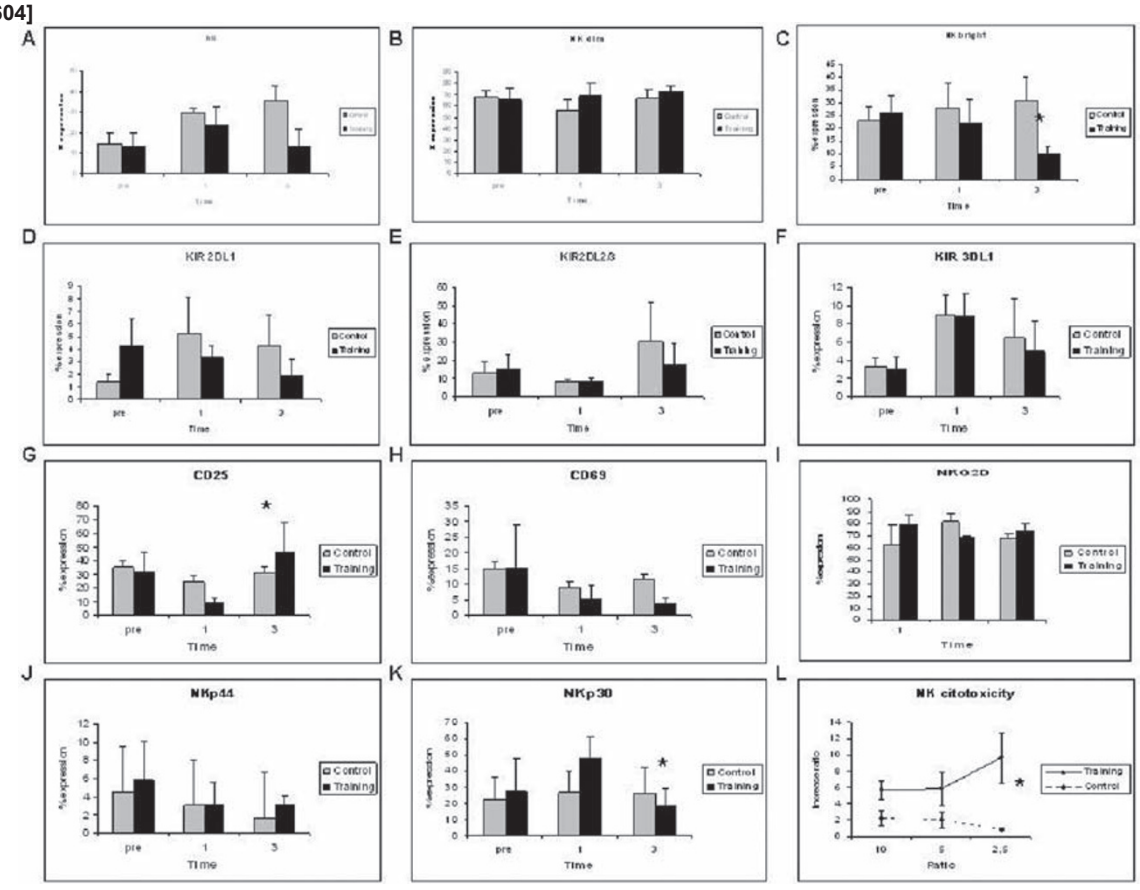

Ad admission he was in good clinical condition except of multiple haematoma. A central venous line was inserted under platelet cover. 8 days late conditioning therapy with busulfan and cyclophosphamid was started. He received cyclosporine A and MTX as GVHD prophylaxis. On day +10 after transplantation multiple haematoma, mikrohaematuria, petechial bleedings and epistaxis appeared. Therefore repeated treatment with tranexamic acid and thrombocytes were acquired. On day +12 thrombocytopenia developed that was unresponsive to platelet infusion. Alloimmunization against platelet membrane GPIIb/IIIa (which was absent before transplantation) was detected. Under the idea of alloimmunization of the autologous cells before transplantation in the course of platelet infusion during Hickman implantation we administered high dosage therapy of immunoglobulin (20 mg/kg, IVIG). Beneath this treatment platelet infusion were successful and bleeding events stopped.
Because of a respiratory and cardial insufficiency due to haemorrhagic lung oedema and fluid overload artificial ventilation was required for 4 days. After haemadilution clinical recovery were observed with engraftment (complete donor chimerism) on day +18. Alloantibodies disappeared on day +25. The patient is currently doing well without bleeding signs or transplantation related morbidity. Donor-antibodies against platelets could not be detected at all measured time points.
In summary, improvement in transplant-related complications with current transplant regimens allows consideration of BMT for life-threatening non-malignant disorders such as selected patients with GT. While it offers a cure, GT carries significance risks, especially the development of anti-platelet antibodies. Therefore platelet transfusions should minimize prior to transplant. IVIG may be helpful in cases of refractory immune thrombocytopenia related to anti-platelet antibodies.

P606
Double cord blood transplantation in juvenile myelomonocytic leukaemia
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Juvenile myelomonocytic leukaemia (JMML) is a rare malignant disease in childhood and can be cured only by allogeneic haematopoietic stem cell transplantation (Allo-HSCT). Non-engraftment or graft rejection represents a significant problem in these patients, especially in the HLA mismatch settings.
A one-year old boy with extreme hepato-splenomegaly was diagnosed with JMML. Hydroxyurea was administered for cytoreduction. Allo-HSCT was planned, and search for a matched unrelated donor was initiated but no living donor could be found. Thus two units of cord blood have been selected with the expectation that the excessive number of immunocompetent cells could cope with the high tumour burden. Both cord blood units (CBU) were mismatched at HLA-A and HLA-DRB1 loci (4/6) to the patient, and at the same time the two units were mismatched to each other at one HLA-A and two HLA-DRB1 loci (3/6).
A conditioning regimen according to the BuCyMel scheme (busulphan 4x0,8 mg/kg for 4 days, cyclophosphamide 60 mg/kg for 2 days and melphalan 140 mg/m² one day) with rabbit antithymocyte-globulin (2,5 mg/kg Thymoglobin for 4 days) was administered. Both cord blood units were transfused on the same day without complications. The nucleated cell, CD34+, CD3+, CD19+ and Treg cell contents were $12{,}6x10^7$/kg, $0{,}27x10^6$/kg, $0{,}15x10^8$/kg, $0{,}06x10^8$/kg and $1{,}3x10^5$/kg for CBU1, and $10{,}3x10^7$/kg, $0{,}22x10^6$/kg, $0{,}16x10^8$/kg, $0{,}05x10^8$/kg and $2{,}8x10^5$/kg for CBU2, respectively. The total nucleated cell (NC) dose was $23x10^7$/kg.
Severe acute graft versus host disease (GvHD) developed early on day +12 in deep aplasia (skin grade III, liver and gut grade I, with cytokine storm and pneumonitis necessitating admission to the intensive care unit), which required 10 mg/kg methylprednisolon to be controlled. During the aplastic phase slow resolution of hepato-splenomegaly could be observed. Engraftment occurred on day +37 with full donor chimerism (0% CBU1, 100% CBU2, 0% recipient). Through the four months follow-up time no major adverse event occurred, the patient remaining in complete remission with no sign of GvHD.
According to our knowledge this is the first published double cord blood transplantation in JMML. We conclude that a high stem cell dose consisting of two units of cord blood is a reasonable therapeutic approach in JMML even for children with very high tumour burden to cope with.

P607
Mutual flutter of serum thrombopoietin levels and platelet counts in the management of thrombocytopenia in children undergoing peripheral haematopoietic stem cell transplantation
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Background: Delayed platelet recovery is a well known complication in patients undergoing either autologous or allogeneic hematopoietic stem cell transplantation (HSCT). In the regulation of megakaryocyte proliferation, thrombopoietin (TPO) has been shown to bear a pivotal role in the activation of antiapoptotic and cell maturation pathways.
Objectives: To define the role of the endogenous thrombopoietin in the evolution of the hemopoiesis after bone marrow transplantation and to determine what extent the exogenous thrombopoietin administration accelerates the development of the megakaryocytic line showing the tardiest, most lingering maturation.
Patients: The fluctuation of the endogenous serum thrombopoietin level and the platelet count during peripherial stem cell transplantation was measured in the case of a child treated of chronic megakaryocytic thrombocytopenia for over 10 years. As controls, these parameters of two children concurrently undergoing autologous and allogeneic HSCT were followed.
Methods: Serum and blood samples for whole blood cell counts, serum thrombopoietin levels were collected and cryopreserved twice weekly. Administration of exogenous thrombopoietin (Nplate®) was indicated after engraftment during lingering thrombocytopenic period. Quantitative determination of the thrombopoietin concentrations were achieved by a sandwich enzyme immunoassay technique using an anti-TPO pre-coated 96-well microplate and visualization was performed by a compatible ELISA microplate reader. At the time of transplantation, stem cell samples were collected from the donor mononuclear stem cell suspension, and the percentage of CD34+/CD61+ and CD41+/CD42a+ stem cell subpopulations was determined by flow cytometry.
Results: The fraction of the initial megakaryocytic progenitor stem cell population of the donor stem cell suspensions were 0.36% and 0.06%, 0.1% in the case of the child suffering in chronic thrombocytopenia and the controls, respectively. Over the monitoring period of the transplantations (2 months), mutual shifts of the TPO levels and platelet counts (engraftment > 20.000/microliter) were detected considering the exogenous TPO administration in the balance after engraftment, as well.
Conclusion: Our results have underlied the stimulating effect of the endogenous thrombopoietin level and furthermore, the modulating potential of the exogenous thrombopoietin administration has been also revealed in the thrombopoiesis after stem cell transplantation.

P608

Liposomal Amphotericin B prophylaxis of invasive fungal infections in children and adolescents undergoing haematopoietic stem cell transplantation

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Introduction: Antifungal prophylaxis in pediatric and adolescent patients undergoing hematopoietic stem cell transplantation (HSCT) is not well standardized. Liposomal Amphotericin B (LAMB) has a broad antifungal spectrum and a long terminal elimination half-life allowing intermittent administration.
Patients and methods: In 2009 and 2010, 20 consecutive patients (median age: 11.4 years; m: f=8:12) underwent 23 HSCT procedures (autologous: 12, including 3 tandem transplants; allogeneic: 11) for solid tumors (7), relapsed severe aplastic anemia or myelodysplastic syndrome (5), hematologic malignancies (5), Hurler's disease (2), and Crohn's disease (1). In autologous HSCT recipients, median number of CD34+cells was $7.46x10^6$/kg; leukocyte engraftment occurred on median day +9.5. In allogeneic HSCT, stem cell sources were PBSC (CD34+selected and/or CD3/19 depleted) in 9, BM in 2 patients, from matched unrelated donors (9), matched sibling donor (1) and haploidentical parent (1), respectively, containing a median of $15,37^6x10$ CD34+cells/kg; median day of leukocyte engraftment was +11. Graft versus host disease (GvHD) occurred in 5 patients, of whom one developed GvHD IV (skin+gut). LAMB prophylaxis was started in 2 patients with refractory AML on days -46 and -42, respectively, and in the other patients on median day -3, at a dose of 5 mg/kg at intervals of 3 days (15) or 2 days (8). Patients received a median of 11.5 prophylactic doses until median day + 20.5.
Results: Intervals of LAMB administration were shortened in 8 patients during febrile episodes or CRP increase. Intermittent candida colonization was detected in 9 patients by weekly surveillance cultures. Galaktomannan testing was performed weekly and remained negative in 17 patients, 2 patients had one single positive result each; none of the 19 patients developed an invasive fungal infection during a median follow-up of 10 months. One patient with refractory AML-relapse and GvHD IV on multimodal immunosuppression repeatedly showed positive galaktomannan tests; despite continuous preemptive antifungal therapy with LAMB alternating with caspofungin the patient developed aspergillus pneumonia and died on day +140. LAMB infusions were tolerated well; main side effect was hypokalemia which had to be substituted in 20/23 courses.
Conclusions: LAMB prophylaxis was able to prevent invasive fungal infections in 19/20 pediatric and adolescent recipients of autologous or allogeneic HSCT with tolerable side effects.

P609

Sibling transplant for aplastic anaemia: parvovirus B19 infection in donor doesn't interfere with engraftment

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Introduction: Parvovirus B19 (PV B19) infection affects selectively erythroid progenitors which have a rapid turn over and present a specific receptor. In most of cases the clinical syndrome associated to PV B19 is benign with a prompt antibody response capable of an efficient virus clearance, but occasionally the infection may be persistent.
Case report: This case regards a 14 year-old girl affected by severe aplastic anemia documented by clinical and hematological conditions and confirmed by bone marrow biopsy. Her 9 year-old sister was HLA matched, and so, according to international criteria a sibling bone marrow transplantion (BMT) was scheduled. Pre-transplant screening for viral infection was negative both for donor and recipient, but just two days before the start of conditioning regimen the donor presented a syndrome compatible with PV B19 infection, confirmed by PCR-DNA virus detection. The BMT was stopped and the patient received immunosuppressive therapy based on steroids, cyclosporin A (CyA) and Anti-thymocyte Globulin (ATG), but unfortunately there was no response. The subsequent search of a matched unrelated donor was ineffective. The levels of virus DNA load, monitored both in donor's serum and bone marrow, were pretty stable for about 16 weeks and apparently unmodified by a therapeutic cycle of intravenous Immunoglobulin (IVIG). Only after more than 4 months the viral DNA disappeared from donor's serum and a progressive decrease of viral load was observed in bone marrow. As the patient presented an increasing transfusion support we decided to go on with the transplantation program.
Transplant details are in table 1.
No transfusion support was necessary after engraftment. PCR for PV B19 on BM at day + 60 is negative.
Conclusion: A positive PCR for parvovirus in donor's bone marrow plausibly doesn't affect the engraftment and do not correlate with virus transmission.

Conditoning regimen	Cyclophosphamide 200 mg/Kg
Graft versus Host Disease prevention	Methotrexate + Cyclosporin A
Infection prevention	IVIG + antibiotics
Total Nuclear cells infused	2×10^8/Kg
CD34+ infused	3.4×10^6/Kg
Engraftment neutrophils	Day + 27
Engraftment platelets	Day + 30
Chimerism day +30	Full donor

P610

A child with juvenile myelomonocytic leukaemia and monosomy 7 treated with allogeneic haematopoietic stem cell transplantation and all-trans retinoic acid

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Introduction: JMML that constitutes 1% of the childhood leukemia is a rare disease and fails to respond to chemotherapy. The curative treatment of the disease is allogeneic HSCT and it is fatal at the rate of %90 in the cases who do not undergo HSCT. We present a pediatric JMML case with monosomy 7 in which ATRA used as a differentiating agent after the allogeneic transplantation.
Case Report: A 7 year-old boy admitted to our hospital with ecchymoses on his body. Physical examination revealed splenomegaly, 7 cm below costal margin. The hemoglobin level was 9.8 g/dL, MCV was 92 fL, WBC was 65300/µL and the number of platelet was 22000/µL. The total monocyte number was found to be 24161/µL. Peripheral blood smear showed 13% myeloblast, 36% monocyte and the number of myeloid precursors was 16200/µL. Bone marrow examination showed 16% myeloblast and 40% monocyte consistent with JMML. Cytogenetic analysis of bone marrow displayed monosomy 7. He received induction and consolidation treatment according to the AML BFM 2004 high-risk treatment protocol. After remission was achieved he underwent HSCT from his HLA-identical brother. The product, containing 2.4x106 /kg CD34+ stem cell, was infused after the myeloablative treatment with busulfan (120 mg/m²/day, 4 days) and cyclophosphamide (60 mg/kg/day, 2 days). Trilineage engraftment (erythroid, platelet and myeloid) occurred on days +17, +18, and +28, respectively. Cyclosporine A and methotrexate were utilized as GVHD prophylaxis. ATRA was started (45 mg/m²/day) on day +60 for 15 days and this treatment was administered for once every three months during a year. He is in complete remission for three years after transplantation.
Discussion: In JMML patients, chemotherapy usually fails and there is only one curative treatment option; HSCT. Relapses occur at the rate of 30-40% after the transplantation and these relapses usually appear within two years. Data on the use of ATRA in JMML cases are very limited.

The negative prognostic factors in our patient were the patient's age at diagnosis as he was 7 years old, monosomy 7 and the low number of platelets. These factors posed high risk for the patient. Herein, we present a child with JMML and monosomy 7 who is in remission for longer than two years with the use of HSCT and ATRA.

P611

Bone marrow as a source of haematopoietic progenitors showed less severity of cGvHD in children: report of a single centre in Mexico

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Introduction: Chronic graft versus host disease (cGvHD) is a well defined entity with devastating implications in post transplanted patients; there is controversy about the predisposing factors, being the source of the Hematopoietic progenitors one of them, thus we analyzed the profile of cGvHD in our center.
Methods: During June 2003 to June 2010 forty five pediatric patients underwent allogenic stem cell transplantation, either from identical HLA sibling donor or at minimum 4/6 matched related or unrelated cord blood (RCB/UCB). Nine cases were excluded of the analysis because 8 presented fail to engraft and 1 early death by anaphylaxis. Age of patients ranged between 1 to 18 years, diagnosis comprised malignant and non malignant diseases. Graft versus host disease prophylaxis consisted on cyclosporine until 12 months after transplantation and short pause of methotrexate.
Results: Sources of hematopoietic progenitors were: Bone Marrow (BM) in 75%, RCB in 3%, UCB 19% and BM plus PBSC in 3% of the cases. cGvHD defined by the NHI working group criteria was present in 7 of the 36 analyzed cases (19%). Of this group global scoring was severe only in one case (14%) whereas mild to moderate in rest of cases (86%). All cases were related with previous acute graft versus host disease (aGvHD). Cytomegalovirus infection was detected in 71.4 % of cases with cGvHD within day +16 to +50; female donor sex was not related either with the presence or severity of cGvHD. Treatment of cGvHD was based on Calcineurin inhibitors, combined either with local/systemic steroids, Bronchodilators, Mofetil Micophenolate, PUVA was added in one case with scleroderma. All patients received nutritional, psychological and periodical assessment by specialist in each affected area. All patients with cGvHD are alive and have a good quality of live, integrated to school and work.
Conclusion: In our population most cases of cGvHD were mild to moderate. We are convinced that the most important factor related to this tendency is that we mainly use BM as a source of hematopoietic progenitors; because prophylaxis as well as treatment of cGvHD did not differ from those used elsewhere. Although the number of patients analyzed in this report is small we considered it consistent.

Haemoglobinopathy and Inborn errors of metabolism

P612

Haematopoietic stem cell transplantation for β-thalassaemia major: the French experience

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Objectives: In order to describe the results of allogeneic HSC-transplantation for β-Thalassemia Major in France, a retrospective study of all patients transplanted in France was conducted by the national center for β-thalassemia in collaboration with the French Society of BMT (SFGM) using the European data base PROMISE.
Characteristics of population: Between December 1985 and 2007, the French transplant program included 108 patients with β-Thalassemia Major who received HSC-transplantation. Their median age at transplant was 6.2 years (0.7-32). The median follow-up was 12 years. As liver biopsy was available in only 41% of the patients, stratification according to the Pesaro risk factors was done as follow: class 1, 2, 1 or 2, 2 or 3 and 3. 88 % of the patients were class 1, 2 or 1 or 2. 26% of the patients had splenectomy before transplant. Most of the patients (95 out of 108) received conditioning regimen consisting in Busulfan and Cyclophosphamide. Antithymocyte globulin (ATG) was progressively added over time in order to improve engraftment and 57 patients received ATG as part of the conditioning. All but 12 patients received a graft from an HLA-matched sibling donor (MSD) and 96 out of 108 were transplanted with bone marrow source.
Results: The 10-year probabilities of overall survival (0S) and thalassemia-free survival (TFS) were 88.8 % ± 5.9% and 69.4% ± 8,6% respectively. 13 patients died. 24 patients had graft failure, of whom 11 received a second transplant (6 successfully). After the second transplant TFS increased to 75,9%.
In univariate analysis, Pesaro risk class was associated with overall survival ($p<0.001$) and TFS ($p<0.001$). Use of ATG and more recent transplant (date of BMT after April 1994 = median date of BMT) significantly decreased the risk of graft failure and improved the TFS. Age at transplant impacted on the overall survival ($p=0.003$), and the use of matched sibling donor (MSD) on TFS ($p=0.003$).
In multivariable analysis, factors significantly associated with improved TFS were the use of a MSD, splenectomy before transplantation, Pesaro classification 1 or 2 and transplantation performed after Apr1994. Overall survival remained only impacted by Pesaro classification.
Conclusion: This national study conducted over a 22-years period showed that genoidentical HSC- transplantation for young children using BuCy conditioning and ATG is a valid treatment option for β-Thalassemia Major.

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Intravenous busulfan in young children with thalassaemia undergoing haplo-identical haematopoietic stem cell transplantation from mother

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Hematopoietic stem cell transplantation (HSCT) remains the only curative option for patients with thalassemia. Advances in hematopietic SCT, supportive care and tissue typing techiques have steadlily led to consider this curative approach also for patients who lack matched related donor using mismatched related donor. Preparatory regimen for BMT of patients with thalassemia must achieve two objectives:elimination of the disorder marrow and establishment of a tolerant environment that will permit transplanted marrow to survive to trith.

High-dose busulfan (Bu) combined with cyclophosphamide (Cy) is the preferred preparatory regimen for patients with thalassemia. In the present study, we hypothesized that intravenous Bu is safe and associated with low toxicity, a high engraftment rate, low severe acute or chronic GVHD. Eleven patients with thalassemia major were conditioned with 60 mg/kg hydroxyurea and 3 mg/kg azathioprine from day -59 to -11, fludarabine 30 mg/m² from day -17 to -11, starting on day -10 patients were given weight-based iv. busilvex with targeted dose adjustment (target AUC range, 900-1350 µMolmin) instead of oral Bu, and 200 mg/kg cyclophosphamide, 10 mg/kg Thiotepa, and 12 mg/kg ATG (Fresenius) daily from day -5 to -2. Intravenous Bu doses were based on actual patient body weigh and was administered over 4 consecutive days in 4 divided doses as an intravenous infusion (concentration, 0.6 mg/mL) for 2 hours. No hepatic VOD prophylaxis was given.

Patients received CD34+ mobilized peripheral and bone marrow progenitor cells from mismatched mother.T-cell dose was adjusted to $3x10^5$/kg by fresh marrow cell addback at the time of transplant. Two patients reject their grafts, and 9 showed full chimerism with functioning grafts at a median follow-up of 16 months. None of the 9 patients who showed full chimerism developed acute GVHD and organ toxicity.

These results suggest that maternal haploidentical HSCT is feasible for patients with thalassemia who lack a matched related donor, and the low toxicity profile observed in our study resulted from the use of intravenous Bu and the more conservative target range with therapeutic drug monitoring.

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Purified T-depleted, CD34+ peripheral blood and bone marrow cell transplantation from haplo-identical mother to child with thalassaemia

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Feto-maternal microchimerism suggests immunological tolerance between mother and fetus. Thus, we performed primary hematopoietic stem-cell transplantation (HSCT) from mismatched mother to thalassemic patient without an HLA-identical donor. 31 patients with thalassemia major were conditioned with 60 mg/kg hydroxyurea and 3 mg/kg azathioprine from day -59 to -11, 30 mg/m² fludarabine from day -17 to -11, 14 mg/kg busulfan starting on day -10 were administered orally 3 times daily over 4 days in the first 17 patients,and corresponding dose of busulfan give intravenous in the following 14 patients, and 200 mg/kg cyclophosphamide,10 mg/kg Thiotepa, and 1o mg/kg ATG (Fresenius) daily from day -5 to -2. 23 patients received CD34+ mobilized peripheral and bone marrow progenitor cells; 8 patients received marrow graft selected PBSC CD34+ and BM CD3/CD19 depleted. T-cell dose was adjusted to 2 x 10^5/kg by fresh marrow cell addback at the time of transplant. Both groups received cyclosporine for graft versus host disease (GVHD) prophylaxis for two months post transplant. Two patients died (cerebral EBV lymphoma or CMV pneumonia), sseven patients reject their grafts, and 22 showed full chimerism with functioning grafts at a median follow-up of 43 months. None of the 22 patients who showed full chimerism developed acute or chronic GVHD. To analyze immunohematologic reconstitution, particularly of natural killer (NK) cells, we evaluated 13 thalassemia patients after 20 and 60 days and 1 year posttransplantation with T cell-depleted HLA-haploidentical stem cells. NKs were among the first lymphocytesto repopulate the peripheral blood. At day 160, an increase in primitive BM progenitor cells paralleled small increases in CD41, naive CD41, and thymic naive Th cells. A significant increase in CD41 and CD81 markers paralleled an increase in CD32CD161 NKs, especially with full engraftment.In patients with stable mixed chimerism we observed very low levels of CD3 donor chimerism early after transplant that increased over time, but a stable population of high donor NK cells, suggesting a role of these cells on donor engraftment. Six patients around days 100-150 post transplant experienced a rise in the copy number of EBV DNA. These results suggest that maternal haploidentical HSCT is feasible for patients with thalassemia who lack a matched related donor.

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Myeloablative BMT in young adults with sickle-cell disease: the French experience

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Allogeneic BMT is currently the only curative treatment for sickle cell disease. However, in adult patients, myeloablative conditioning is usually considered as excessively toxic and non-myeloablative procedures have resulted in high rejection rates or frequent mixed chimerism. We report here the French experience of myeloablative BMT in young adults, using a preparative regimen previously successful in children.

Patients and methods: 12 SCD patients 16 to 28 years old were transplanted in 5 french centers between 1999 and 2009. All patients received the same BU-CY +ATG preparative regimen. Reason for transplant included a stroke history in 4 patients, asymptomatic pulmonary hypertension in 1 patient, and frequent vasoocclusive crisis and/or acute chest syndrome in all the others. The median follow-up of surviving patients is currently 36 months.

Results: All patients engrafted (mean time for PMN > $0.5x10^9$/L: 23days) and no rejection was observed. A patient with previous severe CNS vasculopathy and moya-moya, had a massive intracerebral haemorrhage at day 32 after transplant and died. Early toxicity included also a sub-dural haemorrhage successfully evacuated in a patient without any previous cerebral vasculopathy. Other early complications included seizures, viral pericarditis, bacteriemia and CMV reactivations, but no veno occlusive disease was observed. Four patients had acute GVHD grade II that responded to Prednisone treatment. Two patients had limited chronic GVHD, one with severe peripheral thrombocytopenia; however, both episodes resolved and the patients do not currently receive any immunosuppression. Only one death was observed, resulting in an overall survival and disease-free survival of 92% at 3 years. All survivors have the Hb electrophoretic profile of their donor and a normal Hb level. DNA-chimerism was characterized as full-donor.

Conclusion: This experience demonstrates that myeloablative allogenic BMT is feasible in selected young adults with

SCD: The overall results are similar to those obtained in younger children. A stable long-term full chimerism and cure has been achieved in all survivors. Although this experience is limited, it suggests that myeloablative BMT could be proposed to young adults who have an HLA identical sibling and had non-severe disease during childhood but developed severe criteria during adulthood such as a tricuspid regurgitant jet velocity at least 2.5 m/second.

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Haematopoetic stem cell transplantation in β thalassaemia: Turkish experience

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Nearly half of the thalassemia transplants have been performed in the same center (Pesaro, Italy) which is highly specialized on this topic. It is important to document national data of the countries like Turkey where β thalassemia is more prevalent. Here, we present the results of HSCT for β thalassemia patients on behalf of Turkish Pediatric Stem Cell Transplantation Group.

Between Jan 1991-June 2009, 245 children with β thalassemia major who underwent first allo HSCT in 9 centers in Turkey with a minimum one year follow-up were enrolled this study. Male/Female ratio was 129/116 and the median age was 6,6 (range 1-22 years). Median ferritin level was 2203 ngr/ml, ranging from 74 to 12084 ngr/ml. Forty-one of patients were in Class I, 137 were in Class II, 63 were in Class III and it is not available for 11 patients. Stem cell sources were bone marrow in 88, peripheral blood in 137 and cord blood in 20 transplantations. All donors were HLA matched related donors. Conditioning regimens consisted of BU + CY in 95, BU+CY+ATG in 100, Pesaro Protocol 26 in 40, BU+CY+ Tio-Thepa (TT) in 3. Patients received CsA alone or in combination with MTX or metylprednisolon for graft versus host disease (GvHD) prophylaxis.

The median time to achieve absolute neutrophil recovery was 15 days (range 9-40 days) and platelet recovery was 20 days (range 7-92 days). Median engraftment times were earlier in PBSCT patients compared to BMT group, 13 to 16 days for neutrophil and 17 to 24 days for platelet recovery, respectively ($p<0.001$).Median follow-up period after HSCT was 61 months, ranging from 14-231 months. Acute GvHD was observed in 42 children, 31 of which had Grade II-IV. Chronic GvHD have occurred in 29 patients, 8 with extended form. Thalassemic reconstitution has been observed in 43 transplantations. Nineteen patients died in the first 100 days and transplantation related mortality (TRM) was found as 7.75%. Distributions of TRM according to risk classification were: Class I: 9.75%, Class II: 7.69% and Class III: 6.35%. Seventeen patients were lost after 100 days. Infections, GVHD and bleeding were prominent causes of mortality. EFS (thalassemia free and alive) and OAS were 68,0% and 85.0%, respectively.

In conclusion, collecting national data from different countries in which β thalassemia is more frequent provide management of this group of patients more efficiently. The results of this study confirm that HSCT can offer cure for thalassemic patients.

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Favourable balance of allo-SCT at a long-term follow-up for patients with β-thalassaemia from countries with limited resources

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Allo-SCT from a matched family donor (MFD) offers a hope for cure to β thalassemia patients (Bthal pts) from countries with limited resources. Between 2005 and 2009, 58 consecutive Bthal pts underwent SCT from a MFD at our center (56 from an HLA-identical and 2 from a pheno-identical family donor). Median age 8 years (2-17), origin: Lebanon (9), Iraq (19), Palestine (3), Syria (22), Egypt (2), other (3). Two pt were in class I, 28 in class II and 28 in class III. Class I-II pts were conditioned with ivBu-Cy200 mg/kg (+TT 10 mg/kg if <4 years). Class III pts were conditioned with ivBu-Cy160mg/kg-Flu100 mg/m² or, from April 2007, ivBu-Cy160 mg/kg-Flu150 mg/m²-ATG7.5 mg/kg (Thymoglobuline®) (Chiesa et al, BBMT 2010). GVHD prophylaxis consisted of CyA and short Mtx (+ methylprednisolone for 30 days for pts not receiving ATG). The source of stem cells was unmanipulated BM. Fifty-four pts engrafted donor cells. Four class III pts had primary graft failure, among these 2 died in aplasia and 2 had autologous reconstitution (1 cured by 2ndSCT, 1 chose medical management). The incidence of acute GvHD II-IV was 8/54 (5 grade II, 2 grade III, 1 grade 4) and mortality 1/54 evaluable pts. Six pts had secondary graft failure and were all rescued by 2ndSCT. Nine pts developed mild chronic GVHD, among these 2 had vitiligo as only manifestation. Pts were discharged to their country between 6 and 12 mo following SCT. A follow-up phone contact at a median of 32 months from allo-SCT (11-61), evidenced that one Iraqi patent experienced late graft rejection and died of cardiovascular failure due to lack of transfusion support. All other pts are alive with 100% Lansky score and have returned to school. With the exception of non-resolved vitiligo, only 2 pts, transplanted from the pheno-identical mother, currently off chronic immunosuppressant, still have signs of chronic GVHD evidenced by xerostomia, atrophy and ulcers of the buccal mucosa. Overall survival is 93% (96% in class I-II, 89% in class III), current thal free survival 91% (96% in class I-II pts and 85% in class III).

In conclusion, in Bthal pts from countries with limited resources discharged home 6-12 mo post SCT at a long term follow-up the risk benefit ratio of allo-HSCT is still in favor of transplantation. It is particularly beneficial to offer allo-HSCT from a well MFD to pts with less advanced disease, as the incidence of mortality and graft failure following a myeloablative condition is particularly low.

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Outcome of haematopoeitic stem cell transplantation for RAG1/2-deficient severe combined immunodeficiency or Omenn syndrome

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Objective: Omenn Syndrome (OS), a combined T and B cell deficiency due to “hypomorphic” mutations in genes usually associated with SCID, particularly RAG1/2, is generally classified as a T lymphocyte deficiency within the group of “non-SCID PID”1. Outcome of HSCT for SCID is better than that for other PID2. We compared outcome of HSCT for OS with RAG1/2 SCID.

Methods: Analysis of outcome in patients with OS or RAG1/2 SCID from data submitted to the European registry between 1995-2005. The product-limit method estimated cumulative survival; the log-rank test compared survival between groups.

Results: There were data on 11 SCID, 24 OS between 1995-99, 34 SCID, 20 OS between 2000-05. There were no significant differences between recipient/donor distribution or transplant characteristics in each time period. In 1995-99, SCID patients were transplanted at an older age than OS patients ($p=0.04$). In each time period, survival was similar for SCID and OS, although overall improved in 2000-05.

Conclusion: Whilst outcome of HSCT for SCID is overall better than for other PID, and results are improving, it is surprising that the outcome of HSCT for OS is as good as that for T-B-NK+ RAG-deficient SCID. This suggests that the molecular defect may have an impact on outcome, regardless of phenotype. These results will provide impetus to further understand and

address the problems associated with transplanting patients with RAG deficiency.

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P619

Management of graft failure in MPSI patients receiving HSCT: a single-centre experience

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Mucopolysaccharidosis type I-Hurler Syndrome (MPSIH) is caused by the deficiency of lysososmal enzyme α-L-Iduronidase (IDUA). Allogeneic Hematopoietic Stem Cell Transplant (HSCT) can provide the deficient enzyme by donor derived cells which are capable of delivering enzyme either directly to the deficient cells or by cross correction. In contrast to the malignant disorders where the loss of donor chimerism almost invariably means disease relapse, chimeric states in MPSIH can sometimes deliver acceptable levels of deficient enzymes. We describe our experience of managing primary graft failure in MPSIH patients in this single centre study.
Methods: For monitoring donor chimerism, we use polymerase chain reaction based amplification of short tendem repeats to identify polymorphisms. Patients have their blood samples taken monthly (3 months) and then 3monthly (one year) or more if clinically indicated. Graft function is measured by IDUA assay or Dermatan Sulphate (DS) and Chondroiton Sulphate (CS) ratio. Graft failure is defined as either a donor chimerism level of less 20% or higher with evidence of graft dysfunction eg; rising DS/CS ratio.
Results: Over the last 10 years 52 MPSIH patients received allogeneic HSCT in our centre. Cumulative incidence of graft failure in all patient transplanted between 2001 to 2005 was 26.1% (6/23). Since 2005 this figure is 7% (2/28). In 2005 we changed our transplant protocol which now involves in vivo T cell depletion and administration of busulphan with PK monitoring. 42% received HSCT from matched family donor and 58% from matched unrelated donor. Source of stem cell was cord blood (n=3), bone marrow (n=4) and peripheral blood stem cells (n=1). 75% (n=6) had failed to achieve 100% donor chimerism at 3 months post first HSCT. None of them received donor lymphocyte infusion (DLI). They all received second HSCT with reduced intensity conditioning and T cell depletion and achieved full donor engraftment. Over all long term survival in this group was 75% (n=6).
Discussion and conclusion: In contrast to the malignant HSCT, mixed chimerism in MPSIH does not require donor lymphocytes to generate graft versus leukaemia effect. In our centre results of second transplantation from the same donor, except where a cord donor is used in the first transplant seems a promising approach. It is therefore our practice to monitor donor chimerism closely and use a second transplant instead of DLI for management of graft failure.

P620

Allogeneic HSCT can eradicate allo-immune response to replacement therapy in Lysosomal Storage Disorders and should be considered as a treatment option for immune tolerance induction where inhibitory antibodies render replacement therapy ineffective

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Lysosomal storage disorders (LSDs) are caused by deficiency of lysosomal enzymes. The treatment options include enzyme replacement therapy (ERT), allogeneic Haematopoietic Stem Cell Transplant (HSCT) and substrate reduction therapy. ERT can generate immune response and inhibition of ERT by high titre antibodies (>1:10,000) has been recognised as a significant problem. To evaluate HSCT as an immune tolerance induction mechanism we studied the immune response after exposure to ERT and subsequent to the allogeneic HSCT in Mucopolysaccharidosisl (MPSIH) patients.
Methods: We developed, omptimized and validated IgG ELISA and functional assays to quantify the antibodies and assess their functional nature. We analysed the blood specimens from six MPSI patients before and after exposure to ERT (Aldurazyme 0.58 mg/kg, iv weekly for 3 months) and subsequently on regular intervals after HSCT. The peripheral blood leucocyte α-L-Iduronidase (IDUA) levels were measured to confirm the delivery of enzyme by cellular therapy (post HSCT).
Results: All six patients generated immune response with very high titre positivity (1:130000 serum dilution or higher). Median time (after exposure of aldurazyme) to first positive ELISA test was 25.5 days (range 17-110). Functional assays showed up to 40% inhibition of enzyme in vitro. Median time to maximum immune response was 55 days (range 17-110). Median duration of follow up was 177 day (range 122-213). All recipients of HSCT achieved full donor engraftment. All six patients dropped the antibody titres to less than 1:10,000 within a median period of 101 days (range 42-137) with half of them (n=3) showing no detectable alloantibody response against aldurazyme.
Discussion: The inhibition of replacement therapy is critical in certain illnesses eg; infantile pompe and severe haemophiliac disorders. The immune tolerance induction regimens and pharmacological inhibitor bypassing agents are not only expensive and work slowly but also carry serious risks. High mortality has been reported in CRIM negative infantile Pompe disease patients who generated immune response. Significantly improved outcome of paediatric HSCT offers a viable management option as tolerance induction mechanism in refractory cases.
Conclusion: Allogeneic HSCT offers a treatment option in refractory allo immune disorders where immune response can cause treatment failure resulting in serious clinical deterioration and mortality.

P621

Haematopoietic stem cell transplantation for Wiskott-Aldrich syndrome in Israel

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Introduction: Hematopoietic stem cell transplantation (HSCT) is currently the only curative treatment for Wiskott-Aldrich syndrome (WAS).
Aim: WAS is a rare disorder, affecting about 4 per million live births. Experience with HSCT is available but limited, and outcome data is needed to plan the best treatment regimen. We report data on 13 patients with severe WAS phenotype who underwent HSCT at four Israeli centers from 1996 to 2010.
Methods: Thirteen patients (aged 0.16 to 6.6 years, median 3.25 years) underwent 15 allogeneic HSCTs for treatment of WAS. The conditioning regimens used included Busulfan (Bu) 16 mg/kg + Cyclophosphamide (Cy) 200mg/kg in 6 patients. In one patient Bu 16 mg/kg + Cy 120mg/kg was augmented with Thiotepa. Seven patients were conditioned with Fludarabine-based (Flu) regimens. ATG was added in 8 transplants. Donors included a four matched siblings (in 2 cases from umbilical cord blood (UCB), 2 matched unrelated donors, one matched family donors, 4 unrelated UCB's and 2 haploidentical family donors. Two patients required second transplantations. Follow up through October 2010 ranged from 3 to 130 months.

Results: Twelve of 13 patients are alive with a median follow up of 40 months. Eleven survived with complete clinical, immunologic and hematologic recovery. A 6.5 year old boy who received a transplant from a matched family donor after Flu-Bu conditioning. He experienced primary graft failure and underwent a second transplantation after reconditioning with Cy but unfortunately did not demonstrated engraftment and died two and half months after from multiorgan failure. One patient who underwent haploidentical transplantation demonstrated a mixed chimeric state; he had clinical and immunologic recovery but continued to suffer from thrombocytopenia. Another patient who underwent haploidentical HSCT also developed mixed chimerism; he developed autoimmune thrombocytopenia that was successfully treated with immunoglobulins, steroids and Rituximab. Four patients developed aGvHD grade 2-4 and three of them subsequently developed chronic limited GvHD.
Conclusion: HSCT in children with WAS is associated with a good survival rate. The results from UCBT are comparable with other graft sources, and in this small group of patients demonstrated better results as compared to haploidentical donors. Further studies to determine the best conditioning regimen and optimal alternative donor are required.

P622
Successful treosulfan-based conditioning in patients with inborn errors: a single-centre experience
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Objective: To examine the feasibility, safety and efficacy of a preparative regimen with treosulfan/thiotepa/fludarabine in patients with inborn errors given allogeneic marrow transplantation at a single center.
Patients and methods: the 7 patients enrolled (4 males and 3 females with a median age at transplant = 1 year, range 0,2-16 years) were transplanted for SCID (n=2), malignant osteopetrosis (n=2), Wiskott-Aldrich syndrome (n=1), Severe Congenital Neutropenia (n=1), and Diamond-Blackfan Anemia (n=1). Donors were HLA-matched sibling donors (MSD) for 2 patients, partially matched family donors (PMFD) for 1 patient, and matched unrelated donors (MUD) for 4 patients. The source of hematopoietic stem cells was bone marrow in 4 cases, peripheral blood in 1 case and cord blood in 2 cases. Cytoreduction included Thiotepa (8-10 mg/Kg on day -7), Treosulfan (14 g/m^2 from day -6 to -4) and Fludarabine (40 mg/m^2 from day –6 to -3). In very young children of less than 10 Kg body weight, doses were opportunely adjusted for weight. GVHD prophylaxis consisted of cyclosporine A and short methotrexate (MSD) ± anti-thymocyte globulin Fresenius in MUD HSCT. The patient transplanted with the G-CSF mobilized, CD34+ selected PMFD graft received antithymocyte globulin alone, without post-transplant pharmacological GVHD prophylaxis.
Results: The early HSCT course was uneventful and all 7 patients engrafted with full donor chimerism (>95% donor cells). PMN and PLT take occurred at a median of 20 days (range 11-42) and 27 days (range 8-64) after HSCT, respectively. There was no veno-occlusive disease (VOD), and 3 patients experienced self-limiting grade 2 toxicity. Only one patient developed grade II skin graft-versus-host disease (GVHD) successfully treated with steroids. All patients are alive and disease-free, with a median observation time of 200 days (range, 120–300).
Conclusion: This regimen was well-tolerated, with limited toxicity, efficacious, and with no evidence of rejection and only minimal GVHD. For these reasons this treosulfan-based pretransplant preparative regimen might find elective application in very young patients with inborn errors.

P623
Haematopoietic stem cell transplantation for primary immunodeficiency disorders: The King Hussein Cancer center experience
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Purpose: To evaluate the outcome of children with Primary Immunodeficiency Disorders (PID) who received Hematopoietic Stem Cell Transplantation (HSCT) at King Hussein Cancer Center (KHCC) in Amman, Jordan.
Patients and methods: Retrospective chart review of all pediatric patients less than 18 years of age with PIDs who received HSCT at KHCC between August, 2003 and August, 2010 was performed.
Results: Out of 300 pediatric patients who received HSCT at KHCC, 21 patients had PID. Thirteen were males and eight females. The median age was 1.5 year (3 months -11 years). Eleven patients (52%) had severe combined Immunodeficiency (SCID), and 10 patients had non-SCID immunodeficiency that include: Chédiak–Higashi Syndrome (n=3), Wiskott-Aldrich Syndrome (n=2), Griscelli Syndrome (n=1), Familial Hemophagocytic LymphoHistiocytosis (n=1), Autoimmune lymphoproliferative Syndrome (n=1), Omenn's Syndrome (n=1) and unclassified combined Immunodeficiency Syndrome (n=1). Thirteen patients (62%) received matched-related HSCT, Five patients (24%) received haplo-identical HSCT, and three patients (14%) received unrelated cord blood transplantation. Ten patients (47%) didn't received conditioning (all were SCID), 9 patients (43%) received myeloablative regimens, and 2 patients (10%) received reduced intensity conditioning.
After a median follow up of 46 months (4-86); 16 patients (76%) are still alive. Five patients died; causes of death were variable (CMV pneumonia, liver failure, sepsis, and acute GVHD). Five patients (24%) had BCGiosis at presentation or following HSCT. Twelve patients (52%) had CMV reactivation following HSCT, one of them died with CMV pneumonia. Five patients (24%) required a second HSCT due to primary graft failure, and one patient required a third transplantation. The estimated 5 year overall survival was 76% for all patients, 82% for SCID patients and 70% for non-SCID patients.
Conclusion: HSCT for PID can be performed in developing countries with acceptable results. We have unique issues at our area such as delayed diagnosis and referral to transplant centers, BCGiosis, inadequate genetic tests and limitation of unrelated donor and cord blood HSCT.

P624
Bone marrow transplantation for sickle-cell disease: the first Brazilian cases
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In Brazil sickle cell disease (SCD) is the most frequent inherited monogenic disease. Data from the neonatal screening program showed that about 4% of the brazilian population harbors the sickle cell trait, reaching until 10% in the afro-brazilians. In the northern states of Brazil Bahia 1/17 births are of children with sickle cell trait. The neonatal screening program doesn't cover the whole country so the estimate of about 3500 newborns with SCD per year in Brazil is certainly underestimated. Hydroxiurea (HU) is already used in more severe forms of SCD in Brazil SCT is however the only available curative treatment for the disease. Here we describe the first 9 brazilian cases transplanted in severely affected patients. Median age 16 years (3-38), all donors HLA-id siblings, 2 sickle cell trait. Indications consisted of acute chest syndrome, priapism, alloimunization and silent cerebral infarctions and stroke.

Conditioning regimen was BuCy or FluBu in all but 2 patients who received FluCy (38 years old grade IV liver fibrosis and girl with Moya-Moya syndrome). All patients engrafted, but one patient conditioned with FluCy lost the graft about 100 days after transplant. She received a second transplant 3 years after with BuCy from the same donor, had an 100% engraftment without any sign of GVHD but unfortunately died 3 years later in remission from a SNC bleeding caused by her acquired vascular abnormality. Median follow up for all 9 patients is 1 year (152 days - 7,8 years). Overall survival is 8/9 death cause described above. Acute GVHD grade II were observed in 2 patients (gut and skin) easily treated with short time prednisone. All patients but one are full chimeras and the 38 years old patient remain a stable mixed chimera 5 years after transplant. Three more patients with SCD were transplanted in other centers in Brazil, all of them well and alive some for more than 8 years after transplant. These data confirm the data from other countries and reinforce us to offer this curative approach to patients not responding to HU. The dead of our patient with Moya-Moya syndrome should encourage brazilian pediatricians and clinicians to consider SCT earlier in the disease before irreversible organ damage install. Based on these preliminary positive results a prospective trial is planned in Brazil who should address also long term toxicities and quality of life. Most patients transplanted so far refer as extremely grateful been free of pain.

P625

Haematopoietic cell transplantation for Hurler's syndrome. Improved results in the laronidasa era

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Objective: Hurler´s syndrome (HS) is a mucopolysaccharidosis due to a deficiency of lysosomal α-iduronidase (IDUA), which degrades glycosaminoglycans (GAGS). Hematopoietic stem cell transplantation (HCT) is the treatment of choice in these patients. Graft failure, increased frequency of infections and organ toxicity still can limit the success of this procedure. Enzyme replacement therapy (ERT) with IDUA is another treatment for HS. ERT after HCT might ameliorate clinical symptoms of HS and could result in less transplantation-related morbidity and mortality and better stem cell homing, although information using this approach is limited.
Methods: Four patients with HS underwent to 5 consecutive HCT. All of them were female: median age 14 months (m) at diagnosis (range 12-37 m) and 31 m at the time of HCT (range 24- 51 m). All of them were given ERT with IDUA from diagnosis to HCT and subsequently until endogenous production > 10% normal range was documented. One patient was re-transplanted because of late graft failure (13m after). All cases were from unrelated donor (UD), but one from HLA identical sibling. Source of stem cell was umbilical cord blood in 3, median total nucleated cells infused: 6.4 x 10^7/kg and bone marrow in 2 cases. Conditioning consisted on intravenous busulphan at myeloablative doses adjusted by weight, plus cyclophosphamide 200 mg/Kg. Thymoglobuline was added in UD-HCT. Immunoprophylaxis consisted on cyclosporin, plus methotrexate in case of UD.
Results: All patients are alive and engrafted, median follow-up after HCT of 22 months (5 to 45). Donor chimerism > 95% was reached in all patients. One patient was successfully re-transplantated with a second UD. Among the 5 HCT performed, the median time to get neutrophils > 1000/mm³ was 17 days (range 12 to 20 days) and for platelet engrafment > 20.000/mm³ was 20 days (range 10 to 25 days). ERT was maintained affter HCT without significant related side effects (range 4 to13 m). Acute GvHD (> or = grade 2) appeared in 2/5 patients (40%). No chronic GvHD was noted. Infectious complications were presented in 4/5 patients (80%) but they were not severe with the exception of non lethal respiratory syncytial virus pneumonia. Currently all patients are alive, doing well, and have significant slowdown of the underlying disease.
Conclusion: IDUA treatment after a HCT is a well tolerated approach and may improve the chance of success of this procedure in children with HS.

P626

A novel syndrome of congenital sideroblastic anaemia, B-cell maturation arrest with hypoγglobulinaemia and recurrent inflammatory illness successfully treated by allogeneic unrelated donor BMT

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A male caucasian child (of non-consanguinous parents) presented at 7 weeks old with fever, irritability and poor feeding. He was anaemic (Hb 8.2 g/dl, MCV 74fl) with normal iron studies and >50% ringed sideroblasts in bone marrow (BM). A diagnosis of congenital sideroblastic anaemia was made. DNA analysis found no evidence of known mutations causing sideroblastic anaemia (ALAS2 or SLC38A25). He then suffered episodes of inflammatory illness at ~4 week intervals with malaise then fever, oedema, raised inflammatory markers, cytopenia and haemodynamic collapse (with negative blood cultures), typically resolving in 3-5 days. Further tests showed hypoγglobulinaemia and low B cell numbers, suggesting an inherited immunodeficiency syndrome. BM immunophenotyping confirmed B cell maturation arrest. Between inflammatory paroxysms lymphocyte counts were normal. Despite regular transfusions, immunoglobulin replacement and prophylactic antibiotics these episodes continued. Investigation of this triad of sideroblastic anaemia, B cell maturation arrest/hypoγglobulinaemia and recurrent inflammatory illness failed to identify any known genetic, molecular or mitochondrial disorder. Communication with colleagues in Europe identified several other children with a similar complex of features, with some reports of fatal neurological and cardiological deterioration.
We performed a matched unrelated donor allogeneic bone marrow transplant (BMT) at 8 months of age. Conditioning comprised IV busulfan, cyclophosphamide (200 mg/kg) and alemtuzumab (1 mg/kg), with cyclosporin A used for graft-versus-host disease (GVHD) prophylaxis. Transplant course was initially uneventful, with neutrophil engraftment on day+11. Grade 1 skin GVHD required short-course corticosteroids. At 3 months post BMT he was admitted with fever and collapse, mimicking his pre-BMT episodes. However, he had full donor engraftment and Enterobacter cloacae was isolated from blood cultures. Full recovery was made with antibiotics and supportive care. Immune suppression was withdrawn at 6 months post-BMT. B cell maturation arrest was no longer demonstrable. At 14 months post-BMT he remains well with normal blood counts, normal development, and no signs of the other features displayed by this stage in other children affected by this disorder. Full donor engraftment is maintained. We demonstrate the successful use of allogeneic BMT to cure the haematological and immunological manifestations of this novel disorder.

P627

Spontaneous recovery of donor engraftment following prolonged low-level donor chimerism following BMT for Kostmann's syndrome

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A 4-week-old male presented in 2002 with disseminated pseudomonal sepsis and profound neutropenia (<0.1x10^9/l). Bone marrow revealed myeloid maturation arrest at the promyelocyte stage, consistent with a diagnosis of Kostmann's syndrome

(KS). Genetic analysis identified a typical ELA2 mutation. His presenting illness was managed with antibiotics and regular granulocyte colony-stimulating factor (GCSF) injections. However, neutrophil response remained suboptimal despite incremental dose escalation of GCSF to 40 mcg/kg/day. He developed splenomegaly and blood features of extramedullary haematopoiesis.
In April 2003 he underwent full-intensity BMT from a matched unrelated donor, conditioned with busulfan, cyclophosphamide, fludarabine and alemtuzumab. Graft-versus-host disease prophylaxis was with cyclosporin A. He received an unmanipulated cell dose of $10x10^8$ nucleated cells/kg, with neutrophil engraftment observed day+13 (D+13). D+26 chimerism confirmed 100% donor engraftment. However, unstable mixed chimerism ensued with donor engraftment falling to 79% at D+100 and 5% on D+124. Autologous reconstitution heralded recurrence of agranulocytosis, requiring further daily GCSF support. For 6 years he maintained a stable low-level donor chimerism of ~5%, with neutrophils $0.5–1.0x10^9$/l on GCSF 200mcg/day. Surveillance marrows showed no clonal evolution/leukaemic progression. However, there has since been a sustained and progressive rise in donor cell engraftment from 2% to >30% in whole blood, and even greater in the neutrophil compartment. This has enabled us to significantly reduce the dose of administered GCSF. Currently neutrophil count is maintained and he remains infection-free on twice-weekly GCSF only.
This report emphasises 2 important points:
1) Decisions concerning 2nd transplant in paediatric nonmalignant disease should not be made on chimerism alone, but rather on the function of the graft. Here, despite very low levels of engrafted donor cells adequate graft function could be maintained by GCSF expanding the small number of donor cells present.
2) Significant recovery of donor engraftment in this manner so late after transplant is very unusual, and suggests that the normal donor cells might have a proliferating advantage over recipient Kostmann cells. We hypothesise that the natural history of KS involves progressive hypoplasia of the host Kostmann stem cell pool, permitting gradual replacement by expansion of persistent engrafted donor stem cells.

P628

Haematopoietic stem cell transplantation for inborn errors of metabolism: the Iranian experience

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Objective: Inborn errors of metabolism are a group of inherited disorders that affect growth and development as well as neurologic and cognitive functions. Infants with metabolic disorders are usually normal at birth but clinical deterioration usually occurs shortly thereafter.
Methods: In this retrospective study, we analyzed the outcome of 15 patients (11 boys, 4 girls) with Osteopetrosis (n=9), Hurler syndrome (n=2), Maroteaux-Lamy (n=2), and Niemann–Pick type B (n=2) who had received HSCT between 2007 and 2010. The median age at transplantation was 25.9 months. Patients were transplanted from HLA-identical sibling (n=3), matched relative (n=5), HLA-haploidentical relative (n=3) and unrelated partially matched cord blood (n=4).The source of stem cell were bone marrow (n=7), peripheral blood (n=4) and cord blood (n=4). All patients received a conditioning regimen based on the use of busulfan in combination with cyclophosphamide. The median numbers of MNC and CD34+ injected were 5.85 x 10^8/kg, 5.27 x 10^6/kg, respectively. Cyclosporine with methotrexate was used as GVHD prophylaxis regimen.
Results: At the present time, 12 patients with median follow up of 15 months are still alive and 11 patients are disease free. During the study, 3 patients died. First one was the patient with Osteopetrosis who underwent haploidentical relative donor. He died of sepsis on +10 days after HSCT. The second one was the patient with Niemann–Pick type B who underwent unrelated partially matched cord blood. He died of sepsis on +12 days after HSCT. The last one was the patient with Osteopetrosis who experienced unrelated partially matched cord blood. He died due to CMV infection on +31 days after HSCT. 4out of 10 patients who achieved complete engraftment, had grade III-IV acute GVHD. They also had favorable response to therapy. There was no evidence of chronic GVHD in patients.
Conclusions: HSCT is the only curative treatment option for some patients diagnosed with IEM. It can be devastating if appropriate treatment is not initiated. Although the results of this study indicate that transplant from HLA-identical sibling and matched other related donor results in long-term survival in all patients, transplantation from haploidentical related and unrelated partially matched cord blood requires more cation.it should be noted that in patients affected by genetic disorders (due to consanguineous marriage), donor selection among other related donors should be carefully considered.

P629

Bone marrow transplantation in Gaucher disease. An alternative to enzyme replacement therapy in a country with limited resources. Experience of the Centre National De Greffe De Moelle Osseuse, Tunisia

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Introduction and objectives: Gaucher disease (GD) is an inherited disease due to enzyme glucocerebrosidase (GC) deficiency. Allogeneic bone marrow transplantation (ABMT) which offers the possibility of replacing the missing enzyme activity was used by the past in few patients worldwide. It's currently replaced by enzyme replacement therapy (ERT) or substrate reduction therapy but it may be still an alternative for patients with severe GD who can not benefit from costly ERT.
Patients and methods: Patients with severe GD received between February 2007 and June 2008 ABMT from HLA sibling donors. Diagnosis was made by demonstrating of typical Gaucher cells in bone marrow smears, and low βGC activity. Splenectomy was performed before ABMT in all patients. Conditioning regimen associated cyclophosphamide (200 mg/kg) and intravenous busulfan (12.8 mg/kg).
Results: Four patients aged between 8 to 17 years were transplanted. Nucleated cells infused ranged from 3, 3 to 4 $x10^8$/kg. All patients engrafted. None patient developed acute or chronic graft versus host disease. No death occurred. At 3 months post ABMT, GC activity normalized in 2 patients and remained normal at the last follow-up with a full donor chimaerism. GC activity increased up to 30% and 40% at one year in the 2 other patients but decreased respectively to 7% at 24 months and to 20% at 27 months. Decrease of GC activity was associated to progressive increase in recipient's cells which reached respectively 92% and 97% at the last follow-up. All patients had normal blood count at the last follow-up.
Conclusion: ABMT in GD is associated to low toxicity. It should be proposed to patients with severe GD who can not benefit from ERT. Graft rejection is a concern. Management of immunosuppressive therapy according to chimaerism study results may improve outcome.

P630

Mixed multilineage chimerism and course of globoid leukodystrophy after allogeneic transplantation of CD3/CD19 depleted haematopoietic cells

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Persistent mixed lymphohematopoietic chimerism has been described after allogeneic hematopoietic cell transplantation (HCT) in patients with non-malignant diseases. In children with leukodystrophy, allogeneic HCT is a potential strategy to re-establish the production of galactocerebrosidase-ß galactosidase within the lysosomal compartment of monocytes/macrophages and subsequent development of donor-derived healthy glial cells. We present a case of a 3 year old patient with late-onset infantile globoid leukodystrophy (Morbus Krabbe), in which an HLA-matched healthy sibling donor could be identified. To reduce the risk for acute and chronic GvHD, CD3/CD19 depleted G-CSF mobilised blood stem cells from the donor were transplanted after reduced-intensity conditioning. The patient was prepared for transplantation with daily infusions of fludarabine on day -7 to day -3 (40 mg/m²), thiotepa on day-3 (10 mg/kg) and melphalan on day -2 (140 mg/m²). Without relevant extramedullary toxicity, trilineage engraftment was observed, accompanied by a dominant donor chimerism in circulating white blood cells and CD34+ progenitors. This led to a significant neurologic improvement for the first 18 months after transplantation.

Unfortunately, a decrease of donor chimerism in FACS-sorted CD14+ monocytes, T cells, CD34+ cells and granulocytes below 50% two years after transplantation was followed by an episode of generalized seizures and a stagnation of neurological improvement. A consequent attempt to increase the level of donor chimerism by repeatedly applying donor lymphocyte infusions was unsuccessful.

Now, six years after allogeneic HCT, the overall donor chimerism within white blood cells has stabilized in the range of 20%. The observed correlation between donor chimerism and neurological function argues for a critical threshold of donor chimerism within the monocyte/macrophage compartment required to maintain clinically sufficient levels of lysosomal enzymes after allogeneic HCT for inborn leukodystrophy. Adoptive cell therapy with donor CD34+ cells might be warranted to maintain these levels after T-cell depleted HCT.

P631

Allogeneic stem cell transplantation in 2 siblings with infantile ceramidase deficiency (Farber disease)

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Objective: Infantile ceramidase deficiency (Farber disease) is a rare lysosomal storage disease with autosomal-recessive inheritance associated with distinct clinical phenotypes. Children with neurological involvement usually die in early infancy, whereas patients without or mild neurological findings suffer from progressive joint deformation and contractures, subcutaneous nodules, inflammatory, periarticular granulomas, a hoarse voice and finally respiratory insufficiency caused by granuloma formation in the respiratory tract and interstitial pneumonitis leading to death in the third decade of life.

Patients: We here report on two siblings with FD. The older sibling (Pt1) was diagnosed at the age of 18 months. He appeared to have pain on passive movements with nine months and beginning flexor contractures at the knees, elbows and interphalangeal joints, subcutaneous nodules and hoarse voice at the age of 12 months. Prior to transplantation, the patient was almost unable to walk, but showed no symptoms of central nervous system disease. The diagnosis was confirmed by determination of ceramide accumulation in skin tissue (86% vs. 20% in a healthy control group). The younger sibling (Pt2) exhibited a hoarse voice soon after birth, subcutaneous nodules and joint contractures at the age of 12 months, but also no CNS disease. The ceramide accumulation in his skin tissue was 80%.

The pretransplant conditioning regimen consisted of intravenous busulfan (16 mg/kg), cyclophosphamide (120 mg/kg) and Fresenius anti-thymocyte globuline (60 mg/kg). Both received T-cell replete bone marrow from a phenotypically identical family donor (Pt1) or a fully HLA matched unrelated donor (Pt2). GvHD prophylaxis was cyclosporin A and short course methotrexate.

Results: Both patients had an uneventful peri transplant course. Engrafment at d+16 and d+18 respectively and full donor chimerism was achieved. The subcutaneous nodules and associated pain had already resolved at d+30. Both had no acute GvHD, but Pt1 experienced extensive de novo chronic GvHD at d+100 and died 18 months later from bacterial sepsis. Pt2 is alive and well without contractures, subcutaneous nodules or neurological symptoms at 3 years post HSCT.

Conclusion: Allogenic HSCT offers rapid symptom relief and provides a promising therapeutic approach for FD patiens without neurological involvement. The lack of therapeutic effectiveness of HSCT to central nervous system disease has not yet been cleared.

P632

Second BMT for thalassaemia major using post-transplant cyclophosphamide only as rejection/GvHD prophylaxis

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Rejection after matched related bone marrow transplantation (BMT) for thalassemia major is not a rare event, occurring in 5% to 30% of BMTs depending on age at transplant and risk group. Previous reports have shown that high engraftment rates after second transplantation can be achieved with aggressive conditioning regimens and substantial mortality (Gaziev et al. Bone Marrow Transplant. 2008).

We present the experience on two patients with thalassemia major initially transplanted at ages 1.6 and 1.7 years who rejected their grafts and were re-transplanted from the same donor after 12 and 13 months respectively. First BMT conditioning consisted of thiotepa 10 mg/kg, busulfan 14 mg/kg and cyclophosphamide (CPM) 200 mg/kg, followed by rejection/Graft Versus Host Disease (GVHD) prophylaxis with prednisone, methotrexate, and cyclosporine. Second BMT was performed from with same conditioning drugs but shifting 50% of CPM (50 mg/kg/day) to days +3 and +4 as a means of in vivo bidirectional allodepletion (Luznik et al., Blood 2010). No other GVHD/rejection prophylaxis was administered.

Results: At a follow up of 126 and 74 days both patients have >90% donor engraftment and are transfusion independent. Total neutropenic (ANC<500/mcL) days were 21 and 20, self-sustained platelet counts >20.000/mcL was achieved on days +26 and +22, total platelet transfusions were 6 and 5 and both needed 2 packed red cell transfusions post-BMT. Both patients had fever associated with neutropenia but no other relevant complication, no subclinical CMV activation or GVHD has been detected to date.

Conclusions: This preliminary experience suggests that post-BMT cyclophosphamide is safe, tolerable and effective in promoting engraftment and preventing GVHD after initial rejection of the same donor in thalassemia major patients.

P633

Allogeneic stem cell transplantation in patients with common variable immunodeficiency

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Common variable Immunodeficiency (CVID) is the most frequent primary immunodeficiency, characterized by hypoγglobulinemia and recurrent respiratory and gastrointestinal tract infections. The presentation of the disease is extremely heterogeneous, ranging from simple or no infections to autoimmune manifestations, lymphoproliferation, granuloma formation, cytopenias, and increased incidence of hematologic and other malignancies. The therapeutic approach is based on immunoglobulin (Ig) substitution, immunosuppressive medication in case of granuloma and autoimmunity, blood or platelet transfusions or cytokines and growth factors in case of cytopenia. The role of allogeneic stem cell transplantation (HCT) as curative approach in CVID has been discussed, but no published data are available yet. We describe a cohort of 4 CVID patients that underwent HCT with peripheral stem cell grafts. P001: 40y, male, 3 year history of CVID, presenting with additional Large granular Lymphocyte syndrome, anaemia, lymphadenopathy, and splenomegaly. P002: 38y, male, 12 year CVID history, showed an additional T-NHL together with cytopenia, lymphadenopathy and splenomegaly. P003: 44y, male, 16 year CVID history, severe pulmonary involvement (bronchiectasis), cytopenia, granulomas, lymphadenopathy and splenomegaly. P004: 32y, female, CVID for 27 years with severe lung involvement (interstitial disease), granulomas, cytopenia, and splenomegaly. All patients received Ig substitution prior to HCT. PBSCT grafts were obtained from matched related (n=3) and one unrelated donor. Conditioning regimens were: fludarabin (F) and melphalan (M) (P004) FM+BCNU (P001-003). GvHD prophylaxis included alemtuzumab (P004) or combined cyclosporine/alemtuzumab (P001-003). No graft failure occurred. Patients with clonal T cell proliferation (P001, P002) showed no residual T cell pathology after HCT, prior enlargment of spleen and lymph nodes was substantially diminished. P003 died 3 month after HCT due to infectious problems. While P001 and P002 showed normal values for T and NK cells two years after HCT, only P002 showed normal B cell subsets resulting in independence of Ig substitution. This indepence resulted in a significant reduction of infections and was associated with a complete donor chimerism. In conclusion, HCT is feasible in CVID patients and can result in an improvement of the immunodeficiency. Nevertheless, further application of HCT needs prior definition of suitable patients.

P634

A prospective outcome study on patients with profound combined immunodeficiency

S. Ehl on behalf of the P-CID study group

Currently, there are no clear treatment concepts for patients with combined immunodeficiency, in particular with respect to the appropriate indication and time point for HSCT. We have developed a protocol for a prospective cohort study, in which natural history data will be collected on patients with profound combined immunodeficiency (P-CID). P-CID is defined on the basis of laboratory evidence of T cell deficiency and clinical criteria (episodes of severe infection or immune dysregulation). This will include patients with “atypical” SCID, but also patients with heterogenous, so far genetically undefined combined immunodeficiencies. At study entry, the local center decides and carefully documents, whether and why the patient undergoes HSCT or not. Patients who are not transplanted are followed up yearly and severe events as well as decisions for secondary HSCT are documented. Patients undergoing HSCT also have standardized follow-up evaluation schedules. All three groups (1° HSCT, 2° HSCT, no HSCT within the 5 year follow-up period) will be analyzed with respect to survival, frequency of severe events (infections, immune dysregulation, HSCT related complications) and quality of life. A concomitant genetic and immunological study will aim at a better phenotypic characterization and elucidation of new genetic causes for P-CID. This first prospective outcome study on P-CID will provide important information on prognosis and treatment decisions in this potentially life-threatening disease. The study aims for recruiting 200 patients with a follow-up of at least 5 years and will start recruiting in April 2011.

P635

Is partial splenic arterial embolization a safe procedure to prevent alloimmune graft rejection in a thalassaemic adolescent with bone marrow aplasia before second allogeneic haematopoietic stem cell transplantation?

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Introduction: Partial Splenic Arterial Embolization (PSAE) is a safe procedure that may be an acceptable alternative to partial or total surgical splenectomy in patients with thalassemia. Transfusion requirements and the spleen size may reduce and immune hemolysis, alloimmune reaction might be inhibited by the spleen with this procedure. We thought that PSAE may be a suitable prosedure after graft failure due to alloimmune rejection and it can be applied before the second transplant.
Case: 16-year-old girl with homozygous β-thalassemia who had received monthly transfusion since 9 months of age and received chelation therapy with desferrioxamine. Prior to first transplant, liver was palpable 3 cm and spleen was palpable 7 cm below costal margin, and the ferritin level was 2283 µg/L. According to Pesaro classification she was in the class 3 thalassemia major group. The conditioning regimen was performed according to Pesaro-26 protocol. As GVHD prophylaxis, the patient received cyclosporine and methotrexate. The total dose of nucleated cells infused on day 0 was 3,4 x 10^6/kg CD34+ cells from full match brother donor. Bone marrow biopsy at day +60 confirmed graft failure. We thought that graft failure due to alloimmune response of the spleen. At day +94 PSAE prosedure was performed. After this procedure transfusion requirements and the spleen size were reduced. After supportive therapy for bone marrow aplasia, the second transplant was performed 156 days after the first transplant from the full match same brother donor with the same regimen. The total dose of nucleated cells infused on day 0 was 4,3 x 10^6/kg CD34+ cells. The neutrophil engraftment was reached at day +21, erythroid at day +40, platelet at day +70. Currently, she is transfusion-independent with full single-donor chimerism after 3 months of the second transplant.
Discusssion: PSAE may be a suitable prosedure after graft failure due to alloimmune rejection and it can be applied before the second transplant.

Early complications / Late effects & quality of life

P636

Medical resource use of two different dosing regimens of palifermin to prevent mucositis in multiple myeloma patients receiving one-day administration of high-dose melphalan

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This RCT aimed to study the efficacy of palifermin when administered in two different dosing regimens in a chemotherapy-only

conditioning setting, to reduce maximum severity of oral mucositis (OM). Tertiary objectives included medical resource use, significant infections and duration of hospitalization. The efficacy of palifermin relative to placebo was investigated with palifermin given either pre/post-HDM or pre-HDM in patients with MM undergoing ASCT at 39 European centres. Daily assessment of OM was based on WHO grades (0/1, 2, 3 or 4) until 30 days post transplant or hospital discharge. 281 patients (mean age 56, ±SD= 8 years) were enrolled; 109 patients were randomized to pre-HDM, receiving palifermin (60 µg/kg/day) iv for 3 consecutive days before HDM and 115 subjects were randomized to pre/post-HDM receiving palifermin on 3 consecutive days before HDM and on 3 consecutive days after ASCT. 57 patients were randomized to receive placebo. There was no statistically significant difference in the primary endpoint maximum severity of OM between placebo and palifermin administered pre/post-HDM or pre-HDM. Severe OM (WHO grade 3 and 4) occurred in 37% (placebo), 38% (pre/post-HDM) and 24% (pre-HDM) of the patients. No difference was observed between placebo and palifermin pre/post-HDM, nor pre-only-HDM for the use of i.v. anti-infective drugs (eg. 75, 77 and 73%). There was no difference in non-opioid drug use, whereas use of opioids was somewhat lower in the palifermin treated arms (eg. 77, 67 and 64%, respectively). The median time to ANC recovery of > 0.5 x 10^9L was 11 days in all treatment groups with higher incidence of febrile neutropenia in the pre/post arm. More significant infections were reported in the pre/post-HDM versus placebo group (eg. 51% and 26%). Diagnosis of infections did not require laboratory verification. TPN was used more (eg. 61% and 40%) and longer (eg. mean days 8 and 4) in pre/post-HDM when compared to placebo. The majority of patients (up to 77%) used blood products during the study with no significant differences between treatment groups. Mean duration of hospitalization, 23 days, was similar across the treatment groups. To conclude, palifermin did not show an effect on OM in the HDM setting, most likely due to the timing interval influenced by the short, one day course of HDM. Consequently, palifermin was not able to reduce the medical resource burden related to HDM conditioning used to prepare patients before ASCT.

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Genetic polymorphism of cytochrome P450 1B1 (C432G) is associated with an increased treatment-related mortality and lower overall survival in patients undergoing allogeneic haematopoietic stem cell transplantation

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Background: The human cytochrome P450 1B1 (CYP 1B1) is a key enzyme involved in the production of reactive metabolites and in the activation of environmental carcinogens. Several polymorphisms were identified in CYP 1B1 gene; four of them are single nucleotide polymorphisms and give rise to amino acidic substitutions. The CYP 1B1 codon 432 polymorphism leads to a three-fold higher 4-hydroxylase activity for the variant CYP 1B1 isozymes than the wild types.
Methods: Here we genotyped in a retrospective study 384 recipients (R) (and their donors (D)) for CYP 1B1 (C432G) expression that underwent allogeneic HSCT for various diseases and analyzed their outcome. Genotyping of CYP 1B1 was performed by real-time PCR.
Results: 170 R (44.3%) were genotyped as homozygous wild-type gene C/C, 157 R (40.9%) was genotyped as heterozygous genotype C/G and 57 R (14.8%) were genotype as homozygous gene mutation G/G. From the 167 D (43.5%) were C/C, 164 D (42.7%) were C/G, and 53 D (13.8%) had a homozygous gene mutation G/G. A homozygous CYP 1B1 gene mutation G/G was found on 18 R/D side (4.7%). Five-year estimate for treatment-related mortality (TRM) and overall survival (OS) were statistically different in genotype C/G- and G/G- R with 33 + 4%, and 49+4% compared to homozygous wild-type gene C/C-R (18+3%, and 59 + 4%, respectively, [p<0.03]), whereas the five-year estimate for relapse rate (RR) was not different between the groups. No differences for five-year estimates for TRM, RR, or OS were seen in R with either genotype C/C-, C/G- or G/G- D. No statistic differences were found in the incidence of acute GVHD grade 2-4 on R- or on D- side with variant CYP 1B1 (C432G) polymorphisms. Surprisingly, the five-year estimate for TRM, RR, and OS were statistically different in homozygous gene mutation G/G on R/D site with 57 + 2%, 81 + 2%, and 16 + 1% compared to all other CYP 1B1 genotypes with 28 + 3%, 26 + 2%, and 55 + 3%, respectively [TRM, p<0.01; RR and OS, p<0.001]). Multivariate analysis confirmed that CYP 1B1 (C432G) homozygous gene mutation G/G on R/D site had an increased risk for TRM and RR (p<0.02), whereas the mutation G/G on R/D site revealed a worse OS (p<0.01).
Conclusions: These results suggest that recipients with genetic polymorphism of CYP 1B1 do have an increased TRM, RR and lower OS after transplantation. Genotyping for CYP 1B1 (C432G) might help to identify patients with higher risk for allogeneic transplantation.

P638

Prognostic significance of pre-transplant C-reactive protein levels in patients undergoing allogeneic stem cell transplantation

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Background: The inflammatory response induced during the conditioning regimen may be related to the outcome in myeloablative allogeneic stem cell transplantation (SCT). We hypothesized that elevated levels of CRP (> 10 mg/l) on day -7 prior to chemotherapy, and on day 0 before infusion of the graft, is associated with the outcome. The present study was undertaken to evaluate associations between clinical baseline parameters and pre-transplant CRP levels, as well as the predictive value of CRP levels on the outcome in SCT.
Patients and methods: During the period from 2000 to 2009 CRP values were available on day –7 and on day 0 in 264 patients and 334 patients, receiving a sibling donor or a matched unrelated donor transplant at the National Danish SCT centre.
Indications for SCT included both malignant haematological diseases (n=219 (day –7), n=275 (day 0)) and benign diseases (n=45 (day –7); n=59 (day 0)). Age at SCT in the total study-population was 26,95 years (0,3 years– 60,3 years), mean (range)).
Results: The mean of CRP levels on day –7 was 11,16 mg/l (1-199), and this increased to 27,67 mg/l (range 1-275) on day 0 (p=0,0001).
Elevated CRP (>10 mg/l) was significantly associated with SCT in patients transplanted in advanced remission (CR>1) (p=0,0004) as well as the use of anti-thymocyte globulin (day 0, p=0,0001), and Karnofsky score below 90 prior to the conditioning (p=0,043). Furthermore, CRP levels were related to the diagnoses (p=0,016). Other factor examined, but not found associated with CRP included Total Body Irradiation, and Busulfan-conditioning.
Overall survival was significantly reduced in patients with elevated CRP on day –7 (p=0,003) and day 0 (p=0,017). The same was found for event free survival (p=0,0041 and p=0,0001, respectively). Treatment related mortality showed significantly higher mortality in patients with elevated CRP on day -7 (p=0,0029) and day 0 (0,014).
There was a trend towards an association between elevated levels of CRP at day –7 and the development of acute Graft-versus-host disease grade 2-4 (p=0.08).
Conclusion: This study showing reduced survival in patients with elevated CRP is in line with the notion that the pre-transplant inflammatory status of an individual as well as the inflammatory response during the conditioning have a major impact on the outcome in SCT.

Further investigations concerning the prognostic value CRP will be of interest.

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Donor cell leukaemia – single-centre and European experience: first results

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Introduction: Leukemia or myelodysplasia in donor cells (DCL) after hematopoietic transplantation is a rare but severe complication. Due to low incidence (about 60 cases reported worldwide) very little is known about pathogenesis and management, although different pathogenetic factors have already been discussed. Therefore the Late Effects Working Party decided to evaluate the experiences with DCL within EBMT centers by a two-phase retrospective multicenter study.
Methods: First short questionnaire was sent to all EBMT centers asking for observed cases of DCL. Patients were identified by center identification code and unique patient number and were analyzed with respect to primary diagnosis, age at transplantation date, time between transplantation and DCL diagnosis. In our center 3 cases of DCL have been observed since 1979. These cases were analyzed additionally as to suspected pathogenetic factors, DCL treatment and outcome.
Results: 305 EBMT centers were contacted, 93 answered and 36 reported 54 cases of DCL from 1998 to 2010. 40 cases were evaluable so far by completed patient lists, whereby 36 patients were transplanted for malignant diseases, particularly AML and CML, and 4 patients for non-malignant diseases. Median age at first transplantation date was 36.5 years [4-68 years] and median time between transplantation and diagnosis of DCL was 53.4 months [2-279 months]. In our center 3 cases have been identified, donor origin of blasts was proven by gold standard molecular methods. Two of them had uncommon cutaneous leukemic manifestations at the time of DCL diagnosis. Patients were transplanted with peripheral blood stem cells for AML in one and with bone marrow for CML in two cases, conditioning regimen intended to be myeloablative in two. Donors were all HLA-identical (two siblings, one unrelated). All patients had at least grade II acute Graft-versus-Host Disease (GvHD) and were treated with intensive immunosuppression, in two extensive chronic GvHD occurred. All had infectious problems, two suffered from severe viral complications. Interestingly, two patients were treated with growth factor. Outcome of DCL treatment is poor, one patient died of septic shock, two were retransplanted: one died of DCL relapse, one is alive 2 months later.
Conclusion: DCL incidence is much higher than expected. As result of the first study phase we identified a sufficient case number to further analyze suspected pathogenetic factors from our single center experience.

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T-cell large granular lymphocyte expansion post allogeneic stem cell transplantation: its association with chronic graft-versus-host disease and graft-versus-leukaemia

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Introduction: Polyclonal or oligoclonal T-cell Large Granular Lymphocyte (T-LGL) expansion is an uncommon disorder generally occurring in patients with viral infections, autoimmune diseases, malignancies, and solid organ or allogeneic hematopoietic stem cell transplantation (allo-HSCT). The clinical significance of finding T-LGL expansion post allo-HSCT is unclear. Aim of our study was to evaluate if there is an association between T-LGL expansion after allo-HSCT and stable chimerism, chronic graft-versus-host disease (cGVHD), recurrence of primary disease, and overall survival.
Methods: A retrospective analysis was done on 46 patients whose data were available undergone allo-HSCT with a median follow-up of 61.4 months (r. 17.8-212.5). LGL expansion has been supposed in case of 1) increasing number of peripheral blood lymphocyte counts>2000 cells/mm³ for at least 3 months, and 2) the predominance of LGLs in the peripheral blood smears. Cases with LGL expansion were confirmed for immunophenotypic profiles (CD2, CD3, CD5, CD7, CD8 and CD57 positive) and T-cell receptor RT-PCR for T-cell monoclonality.
Results: Out of 46 patients, 12 cases (26%) showed LGL expansion after allo-HSCT. The median onset of LGL expansion was 35.9 months (r.12.8-100). In 10/12 patients (83.3%) it was associated with confirmed cGVHD, particularly with cutaneous or pulmonary scleroderma-like cGVHD or Sjogren-like cGVHD. In the remaining 2 cases LGL expansion was documented at a follow-up of 12.8 and 37.8 months when common but not diagnostic signs of cGVHD were present. These patients are in actual close follow-up to document an evolution of overt GVHD. Stable mixed hematopoietic chimerism assessed by quantitative real-time polymerase chain reaction was achieved in most patients (73.7% donor component with a range of 45-100%). None of 12 patients experienced disease relapse at a follow-up of 52.4 months (r.14.6-212.5).
Conclusion: it seems that LGL expansion is strongly associated with cGVHD and with graft versus leukemia effect also in absence of full donor chimerism. We think that pts undergone allo-HSCT presenting LGL expansion need to be monitored carefully for systemic cGVHD. Further study in larger series of pts are needed to evaluate this complex mechanism.

P641

Long-term outcome in patients surviving 5 or more years after allogeneic haematopoetic cell transplantation

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As a noticeable number of long-term survivors after allo-HCT continue to increase, many studies have been initiated clinical evaluations and quality-of-life assessment. In this retrospective single centre study, we purposed to evaluate disease status, the probability of survival and late-transplant-related complications in patients surviving 5 or more years after allo-HCT. Between 1989 and 2005 total 484 patients underwent allo-HCT from their HLA-identical siblings or relatives. Of 194 long-term survivors, 173 (91M/82F) were assessed. Median age at transplantation was 31 years (14-51 ys). Their diagnosis was mainly acute and chronic leukemia (90%). Most of the patients were conditioned with a myeloablative regimen and received 52% PB-derived stem cells. Median follow-up after allo-HCT was 10.2 ys (5.2-20.9 ys). The incidence of acute and chronic GvHD was 46% and 76.3%, respectively. At their last control, 36.9% of them had still finding related with chronic GvHD. Twenty-eight patients with chronic GvHD continued inhaler treatment and was frequently hospitalized due to non-infectious obliterative pulmonary complications. Cataract was developed in 10 patients. We observed avascular bone necrosis in 10 patients. Autologous bone graft was replaced in one of them. Secondary malignancy was detected in 4 patients. The frequencies of the development of dislipidemia, hypertention and type II diabetes mellitus were 34.7%, 20.8% and 7.5%, respectively. Social and marital statuses were given in table 1. Fifteen men (21.2%) had become father to a baby after allo-HCT. The relapse of orginal disease was observed in 26 patients (only six after 5years). We performed a second allo-HCT for relapse (n=4) or secondary malignity (n=1). The estimated probabilities of DFS and OS at 10 and 15 years were 83.2%±2.9%, 72.5%±5.4% and 93.3%±2.1%, 82.5%±5.3%, respectively. In conclusion, mortality due to transplant-related complications and relapse rate are decreasing in long-term

survivors. But long-term allotransplant survivor requires a careful follow-up both for medical and still ongoing social problems.

Variables	n (%)
Median age (range)	31 years (14-53 years)
Pretransplant diagnosis	
Acute leukemia: AML/ALL	70/9
Myeloproliferative disease: CML/other	75/1
Bone marrow failure: SAA/MDS/FA	6/7/1
Lymphoma/Myeloma	2/1
Stem Cell Source	
BM/PB/BM+PB	82/90/1
Intensity of conditioning regimen	
Ablative/Reduced intensity	156/17
Chronic GvHD finding beyond 5 years	64 (36.9%)
Dry eye	57 (32.9%)
BOS or BOOP	28 (16.2%)
Immunosuppressive requirement beyond 5 years	10 (15.6%)
Osteopenia/osteoporosis in female patients	51 (74.4%)
Median Body Mass Index	25 (12-40)
Obesity (BMI>29), n	32 (18.5%)
Low BW (BMI<18.5), n	7 (4.02%)
Marital status	
Marriage at pretransplant	105 (60.7%)
Marriage at posttransplant	28 (16.2%)
Single	37 (21.4%)
Fertility	15 (8.6%)
Social Status	
Active working	51 (29.5%)
House wife	57 (32.9%)
Retired	31 (17.9%)
Student	4 (2.3%)
Relapse (within or beyond 5 years)	26 (15%) (n=20/6)

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Thyroid and parathyroid function in long-term survivors of stem cell transplantation after 10-14Gy total body irradiation

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Thyroid and parathyroid dysfunction are well described complications of irradiation to the neck. In this study we have evaluated the incidence of thyroid and parathyroid dysfunction in long term survivors of SCT attending a dedicated haematology/endocrine follow-up clinic. All patients were transplanted for haematological malignancy and had total body irradiation (TBI) as part of their pre-transplant conditioning regimen.
76 patients (37 female) were investigated a median of 18 years post SCT (range 8-29). The median dose of TBI was 12Gy (range 10-14) given in conjunction with cyclophosphamide. 61 patients were treated for CML and 15 patients were treated for acute leukemia.
21 out of 76 patients (28%) developed hypothyroidism and there were no cases of hyperthyroidism. Among male patients the prevalence was 26% compared to <1% in normal populations. Among women the prevalence was 30% compared to a normal prevalence of 0.1-2%. The median time after SCT that hypothyroidism developed was 13 years but the range was wide at 4.5-24 years.
None of our patients had laboratory evidence of hypoparathyroidism (low calcium with inappropriately non-elevated PTH). 16 patients had a raised PTH level. Of these, 12/16 had a calcium level in the lower end of the normal range (2.16-2.4 mmol/l) making primary hyperparathyroidism unlikely. The majority (11/12) of these patients had low vitamin D levels making secondary hyperparathyroidism the most likely diagnosis.
Of four patients with elevated PTH and calcium levels >2.4 mmol/l, none had convincing evidence of primary hyperparathyroidism. One patient had renal impairment and secondary/tertiary hyperparathyroidism; a second was taking bendrofluazide; a third was vitamin D deficient. A fourth patient was hypocalciuric with a 24 hour urinary calcium/creatinine ratio <0.01; this excludes primary hyperparathyroidism and makes the diagnosis of familial hypocalciuric hypercalcemia likely.
In summary, there was a high prevalence of hypothyroidism (28%) among our patients. Conversely there were no cases of primary hyperparathyroidism or hypoparathyroidism but longer follow up will be necessary to clarify the effect of these doses of TBI on parathyroid function. Secondary hyperparathyroidism was frequent, particularly in association with low vitamin D levels. We conclude that indefinite surveillance is needed to identify thyroid dysfunction after SCT and vitamin D levels should be part of routine annual follow up investigations.

P643

At home autologous stem cell transplantation (n=120) for haematological malignancies. A 10-year single-centre experience

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Background: To analyse the clinical outcomes and the impact on healthcare resources of a home care program in autologous stem cell transplantation (ASCT).
Patients and methods: At home ASCT (since day +1) was offered to all patients with a good performance status, a travelling time to the hospital of less than 60 minutes, and a caregiver available 24 h a day. Patients with lymphoma received BEAC or BEAM, patients with myeloma melphalan 200 and those with leukaemia cyclophosphamide (CY) and total body irradiation (TBI). 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 only in case of complications. The fever was an indication of immediate transfer to hospital. The absence of focal infection or signs of severe sepsis allowed returning home after the initiation of parenteral antibiotics.
Results: 120 patients were managed at home (82 men, 38 women), 26% of the ASCT performed in the last 10 years at our institution. 75 (group A) received BEAC (n=10) or BEAM (n=65), 24 (group B) melphalan, and 13 (group C) CY-TBI, while eight treated with other conditioning are not included in this analysis. The median (range) age (years) was 44 (17-67) in A, 48 (25-65) in B and 35 (20-55) in C (B vs. A, p=0.01; B vs. C, p=0.007). Neutropenia (days) of less than 0.1 x109/L (group A: 7 (3.11); B: 6 (3-17) and C: 9 (5-14)) was longer in C (C vs. A, p=0.002; C vs. B, p=0.01). Fever occurred in 80%, 54% and 69% of patients in A, B and C, respectively (A vs. B, p=0.01), with the start (A:+4 (2-10) B:+8 (4-12) and C:+7 (3-13)) earlier in A (A vs. B, p=0.00006; A vs. C, p =0.03). WHO mucositis grade  2 was observed in 35%, 4% and 31% of patients in A, B and C, respectively (B vs. A, p=0.03; B vs. C, p=0.04). 60% patients in A, 25% in B and 38% in C required two daily visits by nurses (A vs. B, p=0.01), while the physician performed 215 (13.5%) visits in 1585 days spent at home. Sixteen (21%) patients in group A needed re-admission at the hospital and only two (5%) in the rest (p=0.03), both from group B.
Conclusion: Proper selection of patients helps ensure the viability of the program and optimise health resources. Patients treated at home after BEAC-BEAM had a higher incidence and earlier onset of fever, more severe mucositis, and increased need for home care and hospitalisation with respect to the rest of the patients, especially with those receiving melphalan.

P644

Pre-transplant serum hepcidin does not correlate with non-relapse morbidity and mortality after allogeneic haematopoietic cell transplantation in patients with AML and MDS

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Hepcidin is an important regulator of iron homeostasis which is often disturbed in recipients of allogeneic hematopoietic cell transplantation (HCT). We prospectively analysed the impact of pre-HCT serum hepcidin (SH), ferritin (SF) and blood units (UB) transfused on outcome after HCT. Three quotients were generated (SF/UB, SF/SH, SH/UB) in 111 consecutive patients (pts) [58 m/53 f, median age 57 years] with AML (n=85) and MDS (n=26) transplanted at the University of Leipzig from February,2008–January,2010. Donors were matched related (MRD) in 23 (21%) and matched unrelated (MUD) in 88 (79%) pts. Preparative regimen was conventional conditioning (CC) with 12 Gy TBI/cyclophosphamide 120 mg/kg in 41 (40%) and RIC with fludarabin 30 mg/m^2/day for 3 days and 2 Gy TBI in 70 (60%) pts. Median SH and SF were 282 (range 6-1595) and 1869 (range 36-17995)ng/ml respectively. SF >1000 ng/ml was present in 91 (82%) pts. Median UB was 24 (0-95) units. There was a poor linear correlation of SH with SF (r=0.6) and UB (r=0.4). After a median follow up of 16 (5-28) months, OS, EFS, non relapse mortality (NRM), and relapse (RI) were 66%, 53%, 17%, and 30% respectively. The incidence of acute (a) GvHD > grade 2 and Chronic (c) GvHD were 48% and 46% respectively. SH, SF, UB, SF/SH, and SH/UB had no impact on outcome but SF correlated with cGvHD and infections >grade 3 in the first 100 days post-HCT (p=0.02). SF/UB <75% was associated with an improved OS (p=0.06), and EFS (p<0.0005), and lower NRM (p=0.02), and cGvHD (p=0.06). EFS in pts with SF/UB <75% was 60% versus 33% if SF/UB was higher (p=0.01). Also, NRM was 11% if SF/URB was <75% versus 33% if SF/UB was >75% (p=0.002). In the absence of relapse, the probability of one-year survival in a patient with SF/UB <75% is 86% compared to 50% for a patient with SF/UB >75% (p=0.001). Median SF of 1844ng/ml was lower (p=0.003) and median UB of 26 (range 8-95) units higher (p=0.005) in pts with SF/UB <75% compared to pts with higher SF/UB [median SF 2573ng/ml, median UB 19 (range 2-63) units]. SF/UB was not influenced by SH, age, gender, diagnosis, type of conditioning and donor. This SF/UB ratio, if validated, might provide a reliable assessment tool for NRM after HCT. Unlike SF alone, this ratio might reflect the sequel of the often simultaneously disturbed pathways of iron homeostasis as hepcidin regulation, erythropoietic activity, inflammation and iron stores.

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High prevalence of metabolic syndrome and its components in long-term survivors of both autologous and allogeneic haematopoietic stem cell transplantation attending a dedicated Late Effects Clinic

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Introduction: Metabolic syndrome (MS), a clustering of risk factors (RF) for cardiovascular disease (CVD), is reported to occur with increased frequency after allogeneic haemopoietic stem cell transplant (HCT). The prevalence after autologous HCT is not well established. We conducted a cross-sectional study to compare the prevalence of MS & its components in adult survivors of autologous & allogeneic HCT attending a dedicated late effects clinic.

Method: Consecutive long-term HCT survivors (disease-free ≥2 years after HCT) had weight, height, waist circumference, blood pressure (BP), fasting glucose & lipids measured. MS was defined by International Diabetes Federation criteria as obesity & any 2 of raised triglycerides, BP, fasting glucose or reduced HDL-cholesterol.

Results: From May 2008 to December 2010, 28 autologous (61% male) & 50 allogeneic (54% male) recipients were assessed. The dominant autograft indication was lymphoma (25/28) & acute leukaemia for allograft (26/50). Myeloablative conditioning was used in 62% of allografts incorporating total body irradiation in the majority (74%). Any grade acute graft versus host disease (GvHD) occurred in 28 (56%) & chronic GvHD in 33 patients (66%). Autograft recipients were older at time of HCT [median 50 years (range 25-63) versus 40 years (2-63), p=0.01] & at study enrolment [median 55 years (range 28-65) versus 43 years (range 19-67), p=0.01]. Median time since HCT did not differ between the 2 groups [4 years (range 2-14) versus 5 years (range 2-18), p=0.62]. The overall prevalence of MS was 37% & did not differ between autograft & allograft recipients (34% versus 43%, p=0.47). A further 36% & 26% respectively had ≥2 MS components (p=0.44). Individual RF prevalence is shown below in Table 1 with no statistical difference observed by HCT type. A trend to more obesity in the autograft patients was seen. 5 patients (3 autograft; 2 allograft) had established CVD.

Conclusion: While recognising the older age of autograft recipients, we found a high overall prevalence of MS not different between autologous and allogeneic HCT survivors. Individuals with MS are at heightened risk of CVD which typically has a long latency. This cohort may therefore need to mature further for a complete picture to emerge. These data should encourage systematic cardiovascular RF screening in long-term survivors of both autologous & allogeneic HCT and the institution of appropriate preventative measures when identified.

Table 1. Prevalence of metabolic syndrome and its individual components in recipients of autologous and allogeneic HCT

	Total n (%)	*Autologous n (%)*	*Allogeneic n (%)*	*p*
Total	78	28	50	
Metabolic syndrome	29 (37%)	12 (43%)	17 (34%)	0.47
2 or more components	23 (29%)	10 (36%)	13 (26%)	0.44
Individual components of metabolic syndrome				
Central obesity (Waist circumference >= 94 cm for men and >= 80 cm for women or body mass index >=25)	47 (60%)	21 (75%)	26 (52%)	0.06
Elevated fasting glucose (>=5.7 mmol/L or on drug treatment for elevated glucose)	22 (28%)	9 (32%)	13 (26%)	0.60
Hypertension (>=130 mmHg systolic or >=85 mmHg diastolic or on specific treatment)	40 (51%)	16 (57%)	24 (48%)	0.49
Reduced HDL-cholesterol (<1.03 mmol/L in males & <1.29 mmol/L in females or on specific treatment)	26 (33%)	10 (36%)	16 (32%)	0.80
Elevated triglycerides (>=1.7 mmol/L or on specific	43 (55%)	17 (61%)	26 (52%)	0.64

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Improved survival after allogeneic haematopoietic stem cell transplantation in recent years

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We analyzed the outcome after allogeneic hematopoietic stem cell transplantation (HSCT) in different time periods. Patients,

mainly with hematological malignancies (n=953), were divided into four different time periods: 1992-95, 1996-2000, 2001-05 and 2006-09.
During the years many factors has changed considerable regarding patient age, diagnosis, disease stage, type of donor, stem-cell source, genomic HLA-typing, cell dose, type of conditioning, treatment of infections, the use of G-CSF, mesenchymal stem cells, cytotoxic T-cells and home care.
When comparing the last period (2006-09) with earlier periods, we found a slower neutrophil engraftment, a higher incidence of acute GVHD grades II-IV and less chronic GVHD. Relapse incidence has been unchanged during the four periods (22-25%).
Overall survival and transplant-related mortality (TRM) improved significantly in the more recent periods with the best results during 2006-09 with a 100 day TRM of 5.5%. When correcting for differences between the four groups, hazards ratio for survival in the last period was 1.61 (p=0.001) and for TRM it was 0.55 (p=0.003).
Despite older patients, more advanced diseases and more HLA-nonidentical donors, overall survival and TRM improved, while relapse remained unchanged.
To conclude, several improvements taken together have resulted in significantly lower TRM and improved survival after HSCT during the last years.

P647
Graft failure in the modern era of allogeneic haematopoietic stem cell transplantation
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Objective: The present study aimed to identify patients at risk for graft failure (GF) in a modern era of allogeneic hematopoietic stem cell transplantation (allo-HSCT).
Methods: All first allo-HSCT at our centre from 1995 to mid-2010 (n=967) were retrospectively included in the study. Graft failure was defined as either primary GF, i.e. recipients who never recovered from neutropenia (absolute neutrophil count <0.5x10^9/l), or secondary GF, i.e. successful engraftment with subsequent loss of donor cells (<5% donor chimerism) or permanent drop in neutrophils (< 0.5x10^9/l) and/or platelets (<30x10^9/l).
Results: In this study, 54 patients (5.6%) had primary or secondary GF. Nine out of ten GFs were secondary GFs (n=48), and the secondary GFs were in most cases due to less than 5% donor chimerism (n=43). Patients with or without GF did not differ regarding donor and recipient age, sex, sex match, or cytomegalovirus match. Cord blood transplants had an increased risk of GF (18%), compared recipients of bone marrow (GF 6%), and blood stem cells (GF 5%, p<0.01). In multivariate analysis, patients with non-malignant disorders were at greater risk of GF (relative risk (RR) 3.28, p=0.01), when compared to patients with acute leukemia. Furthermore, both non-myeloablative conditioning (NMC) (GF=19%, RR 11.4, p<0.01), and reduced intensity conditioning (RIC) increased the risk of GF (GF=10%, RR 2.64, p<0.01), compared to myeloablative conditioning (GF=3%). Nucleated cell dose was also of importance, and total nucleated cell doses of >12.5x10^8/kg reduced the risk of GF (RR 0.36, p=0.03). Immunosuppression with cyclosporine combined with methotrexate had lower risk of GF (RR 0.19, P<0.001) than other immunosuppressive protocols. Recipients of HLA-mismatched grafts (less than 6/6) also had an increased risk of GF (RR 8.10, p<0.01).
Conclusion: At our centre, patients at risk for GF are those with non-malignant disorders, receiving non-myeloablative conditioning regimens, followed by cord blood or bone marrow transplants with a low nucleated cell dose.

P648
Pulmonary complications after autologous haematopoietic stem cell transplantation: a single-centre analysis
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Pulmonary complications (PCs) occur in 40 to 60% of all HSCT recipients, accounting for considerable morbidity and mortality. Their prevalence is lower in autologous HSCT patients in comparison to the allogeneic setting and varies depending on the lack of uniformity in the diagnostic criteria used. The aim of the study was to retrospectively analyze prevalence of non infectious PCs in a cohort of autologous transplanted patients. From July 2006 to December 2008, 178 consecutive auto-transplanted patients were reviewed: 73 lymphoma, 101 MM, 4 AML. Median age was 58 yrs (range 16-73). The timing of PCs was divided according to onset (pre-engraftment, early and late PCs). Type of PCs was defined according to Afessa criteria (BMT 2001): bronchiolitis obliterans (BO), bronchiolitis obliterans organizing pneumonia (BOOP), idiopatic pneumonia syndrome (IPS), peri-engraftment respiratory distress syndrome (PERDS), diffuse alveolar hemorrhage (DAH). Multiple clinical variables were analyzed to determine their influence on the development of PCs: age, sex, diagnosis, tobacco use, history of lung diseases and infections, number of therapeutic lines before transplant, pretransplantation lung function tests (LFT), prior chest irradiation, disease status at transplant, conditioning, number of infused CD34+cells. Chi square and t student tests were used for univariate analysis and linear regression model for multivariate analysis.
Results: 34 of the 178 pts (19%) developed PCs: 10 (6%) and 24 (13.5%) had infectious and non infectious complication, respectively. Of the 24 non infectious PCs, 12 (50%) were defined as PERDS, 10 (42%) as IPS, 1 as BO and 1 as BOOP. Mortality rate of PCs was 8.8% (3/34); 4 required mechanical ventilation, 12 developed chronic lung diseases. At the multivariate analysis age (p=0.000), uncontrolled disease status at transplantation (p=0.022), FEV1<80% (p=0.011) and CD34+ infused >5x10^6/kg (p=0.007) were identified as independent risk factors for PCs. PERDS and IPS were associated with age (p=0.002), uncontrolled disease status (p=0.002), FEV1<80% (p=0.007), more than 2 previous therapy lines (p=0.022) and CD34+>5x10^6/kg infused (p=0.003). In this retrospective analysis pulmonary complications seems to be strongly associated with age, uncontrolled disease, a reduced FEV1 and CD34+>5x10^6 /kg infused. These data may help in the selection of patients undergoing autologous transplantation in order to limit morbidity and mortality from PCs.

P649
Immune cell subsets in haematopoietic cell transplant recipients associated with graft-versus-host disease or infections
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Background: Failure of hematopoietic cell transplantation usually results from relapse, graft-vs-host disease (GVHD) or an infection. These complications are related to immune reconstitution, however, little is known about which immune cell subset is primarily associated with relapse, GVHD or postengraftment infections.
Patients and methods: We determined blood immune cell (leukocyte) subset counts by flow cytometry on day 28 (n=89), 56 (n=94), 84 (n=93), 180 (n=58) and 365 (n=51) after allogeneic transplantation of filgrastim-mobilized blood stem cells or marrow. Conditioning typically included busulfan (~12.8 mg/kg), fludarabine (250 mg/m^2) and Thymoglobulin (4.5 mg/kg). For each leukocyte subset at each time point we evaluated with

multivariate analysis whether the subset was associated with subsequent death, relapse, GVHD or postengraftment infections.
Results: No subset was significantly associated with death or relapse. Acute GVHD (grade 2-4) was associated with high day 28 counts of several T cell subsets, especially naïve CD4 and naïve CD8 T cells, low day 56 and 84 counts of CD5+ B cells and naïve B cells, and low day 28, 56 and 84 counts of plasmacytoid dendritic cells (DCs). Chronic GVHD (extensive) was associated with low counts of monocytes on day 28 and 56 (preceding clinical manifestations). Viral infections were associated with low day 56 or 84 counts of several T cell subsets, especially naïve CD4 and naïve C8 T cells, and low day 56 basophils. Bacterial infections were associated with low day 56, 84 or 180 counts of activated CD4 T cells, CD5+ B cells, plasmacytoid DCs and basophils. No subset was significantly associated with fungal infections. See Table 1 for details.
Conclusion: Acute GVHD may be caused by naïve T cells, and may impair generation of CD5+ B cells, naïve B cells and plasmacytoid DCs. Chronic GVHD development may be inhibited by monocytes. For viral immunity, naïve T cells and basophils appear to be important. For bacterial immunity, CD4 T cells, CD5+ B cells, plasmacytoid DCs and basophils appear to be important.

Table 1. Leukocyte subsets significantly associated with GVHD or infections.

Outcome	Associated Subset	Measured on day	Cutoff count (per ml blood) for dividing patients into a group with high and a group with low counts**	Relative Risk of outcome for group with high over group with low subset counts***	P value***
aGVHD (grade 2-4)	Naïve (CD45RA$^+$CD11a$^{low/int}$) CD4 T cells	28	0.97	3.49	<0.001
	Naïve (CD45RA$^+$CD11a$^{high/low}$) CD8 T cells	28	0.43	3.02	0.007
	CD5$^+$ B cells	56	1.36	0.17	<0.001
		84	5.72	0.21	0.002
	Naïve (IgD$^+$CD27$^-$) B cells	56	6.26	0.24	<0.001
		84	13.68	0.22	<0.001
	Plasmacytoid DCs (CD123$^+$HLADR$^+$CD3$^-$CD11c$^-$ CD14$^-$CD16$^-$CD19$^-$CD56$^-$)	28	3.64	0.27	0.001
		56	1.63	0.34	0.005
		84	1.43	0.10	<0.001
cGVHD (extensive)	Monocytes (CD14$^+$)	28	584.29	0.30	<0.001
		56	203.58	0.36	<0.001
Viral Infections*	Naïve (CD45RA$^+$ CD11a$^{low/int}$) CD4 T cells	56	0.51	0.24	<0.001
	Naïve (CD45RA$^+$CD11a$^{high/low}$) CD8 T cells	84	0.02	0.15	<0.001
	Basophils (CD123$^+$CD3$^-$CD14$^-$ CD16$^-$CD19$^-$CD56$^-$HLADR$^+$)	56	24.05	0.23	0.001
Bacterial Infections*	Activated (CD25$^{low/int}$) CD4 T cells	56	6.10	0.19	0.010
	CD5+ B cells	84	0.33	0.03	<0.001
		180	1.28	0.15	<0.001
	Plasmacytoid DCs (CD123$^+$HLADR$^+$CD3$^-$CD11c$^-$ CD14$^-$CD16$^-$CD19$^-$CD56$^-$)	56	1.41	0.16	0.004
		84	0.48	0.20	0.001
	Basophils (CD123$^+$CD3$^-$CD14$^-$ CD16$^-$CD19$^-$CD56$^-$HLADR$^+$)	56	17.59	0.30	0.037
		84	11.28	0.11	<0.001

* Day 56-179 infections if leukocyte subset was measured on day 56; day 84-364 if leukocyte subset was measured on day 84; day 180-730 if leukocyte subset was measured on day 180.
** Determined based on ROC curves as the cutoff yielding maximum sum of sensitivity and specificity.
*** Adjusted for covariates known to influence the risk of the outcome.

P650
Efficacy and safety of deferasirox for the treatment of iron chelation following allogeneic stem cell transplantation: preliminary results of CILC670AES04 trial
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Objectives: Iron overload is common in patients (pts) undergoing allogeneic stem cell transplantation (SCT). The aim of this trial was to determine the efficacy and safety of deferasirox in pts with iron overload after SCT.
Methods: Phase IV, open-label, multicenter clinical trial carried out in 18 Spanish Haematology Services. Main eligibility criteria included age ≥18 years old, SCT at least 6 months before inclusion, transfusional iron overload (defined as 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≤400ng/mL; subsequent adjustments were based on SF levels and safety markers. SF was monitored monthly. This abstract includes results of an interim analysis at 6 months of an ongoing study.
Results: From December 2008 to April 2010, 30 pts were enrolled. Twenty-eight pts were included in the present analysis; median age was 46 years (range 20-65). Primary disease states included acute myeloid leukemia (n=14), myelodysplastic syndrome (n=5) and non-Hodgkin's lymphoma (n=4). Pts had received the SCT a median of 12.2 months before deferasirox initiation (range 6.5-32.7). Median SF at baseline was 1588.5 ng/mL (range 900.0-4055.0) (n=26) and median SF after 6 months of deferasirox was 1245.5 ng/mL (range 329-9771.5), resulting in a median change from baseline of -355.0 ng/mL (range -1013.0-6172.9). Three pts completed the study after achieving SF≤400 ng/mL before the 52 weeks of treatment. 2 pts discontinued the study due to haematological relapse, and 1 due to consent withdrawal. At the time of this analysis eleven pts had available safety data at study database: 2 pts had SCr elevation (<2xULN; one was considered to be drug-related by investigator), 2 pts had ALT elevation (1 drug-related); drug-related AEs included gastrointestinal disorders (n=3) and rash (n=1). Serious AEs (SAEs) were reported in 7 pts, with a total of 8 SAEs (3 primary disease progressions, 1 febrile neutropenia, 1 herpes zoster, 1 fever with rinhorrea, 1 leukocytosis and 1 massive acute subdural hematoma); none of them considered to be related to deferasirox.
Conclusion: Preliminary data showed a downward trend in SF with 6 months of deferasirox treatment in post SCT setting, with a safety profile similar to that reported in previous studies.

P651
A single nucleotide polymorphism of Granzyme B gene in the recipient predicts relapse after HLA matched unrelated bone marrow transplantation for standard-risk haematologic malignancies
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Granzyme B (GZMB) is a serine protease mediating the rapid induction of target-cell apoptosis by cytotoxic T lymphocytes and natural killer cells. The Granzyme B gene has a single nucleotide polymorphism (A/G) in its exon 2 which affects secretion of Granzyme B from lymphocytes (Girnita: Transplant 2009). In this study we analyzed the impact of GZMB polymorphism on transplant outcomes in patients undergoing unrelated HLA 12/12 matched bone marrow transplantation (BMT) through the Japan Donor Marrow Program. The GZMB genotypes were retrospectively analyzed in a cohort of 720 pairs of patients with hematologic malignancies and their unrelated donors. In patients with standard risk disease, the recipient the G/G genotype, a genotype expected to produce lower levels of GZMB, was associated with a significantly higher incidence of relapse. However, the recipient GG genotype had no significant effect on overall survival. The recipient GG genotype in patients with standard risk disease remained statistically significant in multivariate analysis. The GZMB polymorphism did not significantly influence the transplant outcomes in patients with high risk disease. These results suggest an association between the recipient GG genotype and higher risk of relapse among recipients with standard risk disease of BMT from HLA matched unrelated donors.

P652

Cardiac biomarkers during and after allogeneic haematopoietic stem cell transplantation

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Background: Anthracyclines and myeloablative preparative regimen (PR) followed by hematopoietic stem cell transplantation (HSCT) represent high risk for development of cardiotoxicity. Current biomarkers can detect preclinical cardiotoxicity with higher sensitivity than echocardiography. The aim of this study was to assess subclinical myocardial changes during HSCT using cardiomarkers – troponin T (cTnT) and N-terminal pro brain natriuretic peptide (NT-pro-BNP).
Methods: The study included 21 patients who received from july 2009 to september 2010 an allogeneic HSCT for acute leukemia (11 patients with ALL, 10 with AML) with the mean age of 32,8 years at HSCT (range: 19-58). Conditioning regimen for ALL included total body irradiation (TBI) plus cyclophosphamide (CY), and for AML included CY plus busulphan. All patients were previously treated with anthracyclines with median cumulative dose 250 mg/m² (range: 150-580). Cardiomarkers were measured before PR and at days 0, 14 and 30 after HSCT.
Results: Before PR, mean plasma NT-pro-BNP value was 147,6±119,5 pg/ml. After HSCT (day 0), a further increase to 2012,7±1837,5 pg/ml was observed. Fourteen days after HSCT, mean plasma NT-pro-BNP slightly decreased to 1335,7±1262,7 pg/ml. Persistent elevations thirty days after HSCT were found in 19 patients (497,5±477,1 pg/ml). Baseline plasma cTnT levels were elevated in 3 patients, in 7 patients at day 0 and 14, and remain increased still in 8 patients on day 30 (0,018±0,029). The differences in NT-pro-BNP before and after treatment were statistically significant (p=0,002), whereas the differences in cTnT were not significant (p=0,2). The mean cTnT concentrations were higher in patients with TBI and mean NT-pro-BNP values were higher in patients with AML, what was associated with pretreatment of higher cumulative doses of anthracyclines (median 300 mg/m²).
Conclusions: Administration of PR followed by HSCT can induce in patients treated for acute leukemias acute neurohumoral activation and myocardial abnormalities. Persistent elevations in NT-pro-BNP and cTnT might indicate subclinical cardiotoxicity and require further follow.
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P653

Allo-PBSCT across ABO barriers: time to blood group conversion vis-à-vis molecular chimerism

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Background: Close to half of alloPBSCT occur across ABO barriers, a viable issue in the era of RIC.
Goal: To assess the temporal relationship between molecular chimerism and conversion of blood group among the different types of ABO incompatibility.
Patients & Methods: Patients with Hematological malignancies undegoing ABO/Rh–incompatible allogeneic PBSCT at TASMC between 1999-2010 were included. To determine engraftment, Short Tandem Repeats (STR) were analyzed by Polymerase Chain Reaction (PCR). ABO/Rh was determined using the Diamed gel-card technique. ABO/Rh was recorded as recipient, donor or mixed type and recipient Type & Screen (T&S) was repeatedly performed as necessary before transfusion. Patients were transfused with recipient-type red cells (RBC) untill a change in blood group was observed.
Results: One hundred fifty nine patients underwent an allo PBSCT between 1999-2010. Ninety were ABO/Rh incompatible. Fifty six patients were eligible for analysis: 18/90 died before STR determination, 5 had no STR result and 11 were lost to follow-up beyond 30 days. Of 56 (40 Male/16Female) with complete data, 7 had bidirectional ABO incompatibility, 25 had a major, 15 minor and 9 patients had Rh incompatibility. Median follow-up was 158 days (R=45 days-4years). 46/56 patients had full donor chimerism at some point during follow-up (median 27 days, R=13-214) and 33/46 (71.2%) converted their blood type at a median of 118 days (25-455 days). 10/56 patients remained mixed chimera at a median of 48 days (R=15-140), and only 2/10 converted to donor type.
30/56 patients (54%) had 100% donor chimerism but only one converted to donor type by D30.
At D100 42/56 patients (75%) had 100% donor chimerism, whereas only 13/56 (23.2%) changed to donor blood type. The delay in blood type conversion to full donor type vs. full chimerism by STR was of median 85 days, (R=24-425 days!).
In addition, neither patient age nor sex influenced time to convert to donor blood type.
However, of 25 major mismatched transplants, 15 (60%) did convert to donor type, whereas of 15 minor incompatible pairs, only 2 (13.3%) and none of the bidirectional mismatch (x2= 13.504, p=0.00117).
Conclusions: A delay in ABO conversion is observed, compared to molecular full chimerism.
In most cases, conversion occurred after day 100.
Having a bidirectional, minor or major ABO incompatibility had a significant impact on conversion to donor blood type.

P654

Autoimmune-like complications post-haematopoietic stem cell transplantation in children with nonmalignant disorders: 10 years of experience in one paediatric BMT centre

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Background: Autoimmune-like complications (AILC) have been reported but are still not well-characterized in post-hematopoietic stem cell transplantation (HSCT), such as autoimmune cytopenias (AICP) and autoimmune hepatitis (AIH). AICP include immune thrombocytopenia (IT), autoimmune hemolytic anemia (AIHA), autoimmune neutropenia (AIN), or a combination of two of these disorders or with pancytopenia. Scleroderma (SD) as a manifestation of chronic graft-versus-host disease (cGVHD) and has clinical and pathological characteristics similar to AIC.
Objectives: To evaluate the occurrence of AILC and its risk factors, prognosis and response to treatment in a series of children undergoing HSCT at our hospital.
Patients and methods: Between 2000 and 2010, 76 patients after HSCT for nonmalignant disorders (NMD) were retrospectively reviewed (39 males, 37 females). Forty-five patients were diagnosed with congenital hemoglobinopathies (HGP), 11 with immunodeficiency, 8 with metabolic disorders, 3 with Fanconi anemia, 2 with aplastic anemia, and 3 with familial hemophagocytic lymphohistiocytosis. Mean age of patients was 10.7 years (1.4-24.9 years); median follow-up was 6.81 years (0.6-11 years). The diagnosis of AILC was made on standard laboratory-clinical evidence.
Results: Twenty-two (29%) patients developed AILC. AICP were the most common autoimmune disorder: 64%; SD: 32%; AIH: 4%. The most common AILC in patients with HGP was AICP: 64%; SD 36%, compared to other NMD, AICP: 62.5%; SD: 25%; AIH: 12.5%. No statistically significant difference was observed between the incidence of AILC in patients with HGP and other NMD, full or mixed donor chimerism, female or male, related bone marrow or related peripheral blood (RPB), related cord blood (RCB) or UCB, with or without splenectomy. A higher incidence was observed in patients with ABO-mismatched HSCT, who developed cGVHD and who had UPB as a source of HSCT: 48%, 69%, and 50%, respectively. Twenty-two patients who developed AILC after HSCT showed good outcome

and was not the direct cause of death in any case. The majority of patients responded to standard immunosuppressive therapy and only one patient underwent a second transplant for resistant pancytopenia.
Conclusion: AILC is a clinically significant complication after HSCT that contributes to morbidity but not to mortality. Patients with ABO-mismatched HSCT, who develop cGVHD and who have UPB as source of HSCT are specially predisposed to this complication.

P655
Bronchiolitis obliterans after allogeneic stem cell transplantation: a single-centre retrospective study of risk factors
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To identify risk factors of BO, we retrospectively analyzed 151 allogeneic bone marrow recipients allografted between 2006 and 2008. The median age is 47 years (4–66 years). The ratio male/female is 2. Sixty four patients (42%) had a MAC and 87 patients (58%) had RIC regimen. Busulfan was used as a part of the conditioning in 45% and total body irradiation in 47%. The source of the graft is BM in 32%, PSC in 57% and CB in 11%. In 50 % of the cases the donor is a sibling. GVHD prophylaxis is CsA associated with MTX in 41% of the cases, cyclosporine alone in 25% and CsA with MMF in 30%.
Patients had pulmonary function test pre transplant and at 3, 6, 12 months after transplantation and then every 6 months. BO was defined according to NIH consensus. NIH definition requires: absence of active infection, decreased FEV1 (<75% of predicted normal), evidence of airway obstruction with a ratio of FEV1 to forced vital capacity <70%, elevated residual volume of air (>120% of predicted normal) or an expiratory chest CT or lung biopsy that reveals air trapping or bronchiectasis.
According to this definition, we found 11 cases of BO with a cumulative incidence at 3 years of 13%. BO appeared after a median time of 16 months (6m-25m). In univariate analysis, recipient age 50 y.o. and above (p=0.003), donor age 50 y.o and above (p=0.05), cGVHD (p=0.05), aGVHD (p=0.04), female donor to female recipient (p=0.001) and reduced intensity conditioning regimen (p=0.08) are associated with BO.
In multivariate analysis, aGVHD (p=0.029) (RR6.5, 95% confidence interval, 1.2%-34.7%) and female donor to female recipient (p=0.028) (RR 5.45, 95%, confidence interval 1.19%-24.8%) are risk factors of BO. All the patients who developed BO where treated for GVHD at the time of onset of pulmonary symptoms. Among the 11 patients, 6 died and BO is the cause of death in 3 patients. The remaining 5 patients are alive with a BO controlled with immunosuppressive treatment.
Conclusion: BO is a rare complication after allogeneic stem cell transplantation according to the consensus criteria. It is tightly linked to GVHD. Female donor to female recipient is strongly associated with the occurrence of this complication. This should be verified on a larger number of patients. A reinforced immunosuppressive treatment may control the progression of the disease and prolong the survival of those patients.

P656
Safety of autologous stem cell transplantation without any cryopreservation or the use of growth factors
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Introduction: Autologous stem cell transplantation (ASCT) after a high dose conditioning is an established treatment modality with definitive indications for many hematological disorders. However, this line of treatment requires many expensive resources, such as freezing of the harvest product in order to maintain cell viability until stem-cell reinfusion and the use of growth factors for the management of neutropenia.
The aim of this study is to demonstrate the feasibility and the safety of the ASCT without any cryopreservation neither the use of growth factors.
Material and methods: 1st Group: 10 patients with Hodgkin's lymphoma were treated with high-dose cyclophosphamide (120 mg/kg), etoposide (2100 mg/m²) and BCNU (400 mg/m²) followed by reinfusion of autologous non-frozen PSC, which had been stored for 72 hours at +4°C.
2nd Group: 22 patients with Multiple Myeloma were conditioned with high dose of melphalan (200 mg/m²) followed by reinfusion of autologous non-frozen PSC, which had been stored for 24 hours at +4°C.
All patients in both groups did not receive any growth factor after the transplant.
Results: Results are shown in Table 1.
Conclusion: We conclude that high-dose chemotherapy with non-frozen peripheral stem cells is safe in terms of haematopoietic reconstitution even without using growth factors.

	1st Group (n = 10)	2nd Group (n=22)
CD34+ cells/Kg	3,66 (2,33-13,22)	3,66 (1,5 - 9,7)
Duration of neutropenia, in days, median (range)	13(9-24)	11(10-14)
Duration of thrombocytopenia, in days, median (range)	12(9-37)	12(8-21)
No. of platelet transfusions, median (range)	1(0-2)	1(0-3)
No. of red blood cell transfusions, median (range)	2(0-8)	2(0-6)
Duration of intravenous antibiotics, in days, median (range)	9(0-15)	4(0-13)
Duration of hospitalization, in days, median (range)	21(15-37)	17(12-30)

P657
Anti-Müllerian hormone as a marker of ovarian reserve in pubertal girls and young women with a history of stem cell transplantation in childhood
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Gonadotoxicity is well known late effect after HSCT (hematopoietic stem cell transplantation), especially when alkylating agents such as Busulphan or TBI (total body irradiation) were used for conditioning regimen. Damage of the ovaries causes follicle loss and usually results in premature ovarian failure (POF). Females, who are long term survivors after HSCT performed during childhood, consequently present with amenorrhoea usually with high level of follicle stimulating hormone (FSH), both indicating ovarian failure. Anti-Müllerian hormone (AMH) is established as a novel marker of ovarian reserve. Serum AMH concentration is independent on menstrual cycle or hormonal therapy. It correlates well with antral follicle count and can be useful as a marker of premature ovarian failure.
In 45 females, transplanted in childhood at our center, serum AMH were measured using enzymatic immunoassay (EIA) – Immunotech (normal range 14.28-48.55 pmol/l). Median age of this cohort at HSCT was 13.4 years (range 4.2–18.1), median age at the time of AMH assessment was 18.3 years (range 12.2 – 36.5). 40/45 patients received Busulphan (16 mg/kg) or TBI (≥ 12Gy) as a part of conditioning regimen.
In 43 of them (95.6%) low or undetectable level of AMH was determined with only 2 patients (both without Busulphan or TBI) who maintain normal AMH concentration and normal ovarian function. 21/24 patients with undetectable AMH had high FSH levels and receive hormone replacement therapy (HRT). 5/19 patients (all transplanted in prepubertal age) with lower, but

detectable level of AMH, had normal menstrual cycle without HRT with normal FSH levels. In those residual ovarian activity is anticipated.
The present data show low and undetectable levels of AMH in the most of females in this cohort. Use of high-dose TBI or Busulphan resulted in severe ovarian damage. Therefore at least in such circumstances fertility preservation through cryopreservation of ovarian tissue or oocytes should be offered prior to HSCT. Chance for successful cryopreservation of ovarian tissue or oocytes after HSCT in subgroup of patients with residual ovarian activity (normal FSH, regular menstrual cycle but lower levels of AMH) must be discussed with centers for reproductive medicine.
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P658
Effect of immunonutrition on nutritional status and therapy tolerance in patients with haematopoietic stem cell transplantation
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Introduction: Adequate nutrition support (NS) during hematopoietic stem cell transplantation (HSCT) improves therapy tolerance and reduces severity of various complications.
Purpose: In this study we estimated in HSCT recipients the influence of standard and modified NS regimens enriched with glutamine and omega-3 fatty acids on therapy tolerance, occurrence of post-transplant complications and their severity.
Materials and methods: We analyzed data from 91 HSCT recipients, 44 of them underwent allogeneic unrelative HSCT, 22 allogeneic relative HSCT, 9 haploidentical HSCT and 16 autologous HSCT. 31 of the patients had acute myeloid leukemia, 19 acute lymphoblastic leukemia, 10 Hodgkin lymphoma, 9 myelodisplastic syndrome, 6 chronic myeloid leukemia, 4 osteomyelofibrosis, 3 non-Hodgkin lymphoma, 3 aplastic anemia and 6 other hematological diseases. Sex distribution was even - 46 (50,5%) men and 45 (49,5%) women. Median age was 32,9 (13-71) years. Control group (n=46) included patients with symptoms of gastrointestinal toxicity and caloric intake lower, then 60% for 3 or more consecutive days. These patients received standard NS regimen consisting of low-bacteria diet and parenteral nutrition. The second group (n=17) received modified NS regimen consisting of low-bacteria diet with parenteral nutrition starting on day +1 after HSCT, the third group (n=28) received modified NS regimen with additional glutamine (Dipeptiven, Fresenius Kabi 0,35-0,43 g/kg/day iv) and omega-3 fatty acids support (Omegaven, Fresenius Kabi 1,5 ml/kg/day or Lipo Plus, BBraun 1,5 g/kg/day iv) starting on day +1. In each group we evaluated nutritional status using anthropometrics (height, weight, body mass index, mid-arm circumference, triceps skinfold) and laboratory data (total protein, albumin, macro- and micronutrients).
Results: We observed different severity of mucositis and aGVHD in evaluated patients groups, less significant decrease of anthropometrics and laboratory data was observed also. Between each groups there were no difference in time of engraftment and 1 year survival.
Conclusions: Patients receiving modified NS with glutamine and omega-3 fatty acids tolerate the conditioning regimens and immunological complications in early post-transplant period better, then patients on standard NS regimens.

	Incidence of mucositis	Mucositis grade I-II	Mucositis grade III-IV	Incidence of aGvHD	aGvHD grade I-II	aGvHD grade III-IV
Control group, n=46	78,2%	63,9%	36,1%	47,1%	62,5%	37,5%
Modified NS, n=17	88,2%	80,0%	20,0%	38,5%	83,3%	16,7%
Modified NS (glutamine, omega-3 fatty acids), n=28	75,0%	90,5%	9,5%	21,9%	96,4%	3,6%

P659
Suboptimal vitamin D levels in pediatric blood and marrow transplant patients
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Objective: The incidence of low Vitamin D (Vit D) levels in blood and marrow transplant (BMT) patients is unknown. Chemotherapy, radiation, poor nutrition, and limited sun exposure may put BMT patients at risk for low Vit D levels. Studies suggest a correlation between suboptimal Vit D levels (25-OH < 30 mg/dL) and an increased risk for cardiovascular (CV) disease, hyptertension (HTN), diabetes (DM), malignancies, and autoimmune disease. BMT patients are at high risk for these diseases, in addition to, bone mineral loss and life threatening infections. Vit D repletion is a proven treatment for bone disease (rickets and osteoporosis) and infections (tuberculosis). We hypothesize that BMT patients are at high risk for having suboptimal Vit D levels.
Methods: This is a retrospective review of 66 pediatric BMT patients at a single institution. This heterogeneous population had a range of diagnoses, preparative regimens (reduced intensity and myeloablative) and donor sources (autologous, allogeneic, related and unrelated). Patients ranged in age from >1 year to 21 years. Serum Vit D levels, performed using mass spectroscopy at Sonora Quest Laboratories, were obtained at various points prior to and following BMT. Levels < 30 ng/dL were defined as suboptimal. Suboptimal levels < 20 ng/dL were further categorized as deficient. None of the levels reflected therapeutic replacement of Vit D.
Results: Sixty eight percent of the Vit D levels were obtained within the first year of transplant. The remaining Vit D levels were 2-10 years post BMT. Overall, 60% of patients were suboptimal and 24% were deficient. The highest incidence of suboptimal Vit D levels occurred within the first year of transplant. At 1 year post BMT, 72% of patients were suboptimal and 36% were deficient. At > 8 years post transplant, 33% of patients were suboptimal. Notably, 47% of patients transplanted for non-malignant disease had suboptimal levels.
Conclusion: Suboptimal Vit D levels occurred in 72% and deficiency occured in 36% of BMT patients within the first year. The incidence of suboptimal levels subsequently decreased, but was still observed in > 33% of patients > 8 years post transplant. These results warrant additional research on the relationship between low Vit D levels and complications of BMT: infection, CV disease, DM, HTN, bone mineral loss, and potentially inflammatory processes such as graft vs host disease. Vit D repletion may limit complications of BMT.

P660
Health-related quality of life assessment during high-dose chemotherapy and stem cell transplantation in patients with haematologic malignancies
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High-dose chemotherapy and hematopoietic stem cell transplantation (HSCT) could potentially be associated with major physical and emotional distress as well as an overall reduction in health-related quality of life (HRQoL). Recently, the European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life Group developed a treatment-specific questionnaire (QLQ-HDC29) to supplement the widely used generic measure of HRQoL in cancer patients (QLQ-C30). This supplementary module may prove to be particularly useful in assessing specific side-effects of treatment or comorbidities in patients receiving auto- or allo-HSCT.
We investigated the feasibility of these questionnaires in a prospective pilot study of 37 consecutive patients with hematologic

malignancies (mean age 49, 18 females and 19 males) treated with auto- (18/37) or allo-HSCT (19/37). Both questionnaires were administered 1 month before (at baseline) and 3, 6 and 12 months after transplantation. One year event-free survival was 76% and 78% in the auto- and allo-group, respectively. The cumulative incidence of acute and chronic graft-versus-host disease (GVHD) in the allo-group was 36% and 21%, respectively. The global HRQoL scale of QLQ-C30 showed a trend toward improvement from baseline to 12 months after transplantation (mean scores 56.6 vs 63.7, P=0.06). Patients with mean hemoglobin values ≥10 gr/dl experienced a significant increase in HRQoL from baseline to 12 months after HSCT (54% vs 89%, P≤0.01). At 12 months post-HSCT, we observed significant worsening in the Gastro-Intestinal Side Effects Scale (P=0.03), the Worries/Anxiety Scale (P=0.05) and Spiritual well-being items of the HDC29 questionnaire. Skin Problems significantly increased at 3 months after transplantation (p=0.03) but subsequently returned to baseline values. Other HRQoL areas (including Body Image, Impact on Family life and aches in bones) did not show significant differences from baseline up to 1 year after HSCT. Finally, 20 patients (54%) returned to their previous occupational activities.
In conclusion, our study shows that the HDC29 questionnaire used in combination with the more generic measure of HRQoL (C30) provides us with a more complete picture of HRQoL in auto- or allo-transplanted patients. Their combined use supplies us with more detail of the patient's perspectives on the effects of treatment and should thus represent a valuable adjunct in the longitudinal follow-up of these patients.

P661
AMH and Inhibin B are valuable markers to identity subfertility in childhood cancer survivors after allogeneous stem cell transplantation
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Background: Childhood cancer therapy is gonadotoxic, resulting in infertility and premature ovarian failure. Currently, serum AMH levels and inhibin B levels are the most sensitive markers of gonadal reserve in females and males, respectively.
Materials and methods: Serum AMH and inhibin B levels were measured 500 childhood cancer survivors and compared with controls.
Results: 182 female childhood cancer survivors and 248 male childhood cancer survivors were included. In females, median age at diagnosis was 6 years (range 0-17 years). Median age at time of follow-up and assessment of AMH levels was 26 years (14–47 years). Median interval between treatment and AMH assessment was 18 years (5–43 years). In males, median age at initial diagnosis was 5 years (range 0-15 years). Median age at follow-up was 23 years (range 18-41 years) with a median follow-up time of 18 years (range 5-39 years).
In the total cohort of female survivors, AMH levels were within normal ranges of age-matched controls (P=0.57), whereas in male survivors serum inhibin B levels were significantly lower than in controls (P<0.001). Using AMH and inhibin B we identified three groups at risk for gonadal failure, based on previous treatment regimen: survivors which received total body irradiation, three or more procarbazine containing chemotherapy cycles, or abdominal or gonadal irradiation.
Conclusions: serum AMH levels and serum inhibin B levels can be used to identify subsets of childhood cancer survivors at risk for gonadal damage. Therefore, we recommend that children and their parents should be counseled for fertility preservation techniques prior to the start of these types of treatment.

P662
Prevalence of metabolic syndrome and insulin resistance in women after haematopoietic stem cell transplantation
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Introduction: The clustering of central obesity, arterial hypertension, hyperglycemia and dyslipidemia, known as the metabolic syndrome (MS), is associated with an increased risk for type 2 diabetes mellitus and cardiovascular disease. The prevalence of MS is suspected to be higher among hematopoietic stem cell transplantation (HSCT) recipients, but the factors associated with its development remain not well defined.
Objectives: To compare the prevalence of MS and insulin resistance (IR) in 113 women survivors of HSCT and 48 controls and to describe the factors associated with MS and IR in eligible patients.
Methods: All patients gave written informed consent before enrollment. We performed a cross-sectional single center study. The variables analyzed were: MS components on the basis of the NCEP-ATPIII criteria and IR by the homeostasis model assessment insulin resistance index (HOMA-IR), adjusting for transplant type, graft-versus-host disease, hypothyroidism, hypogonadism, hormone therapy, immunosuppressor use, age, age at transplant, time since transplant, MS diagnosis before HSCT, conditioning regimen, race, menopausal status, smoking habits, body mass index, diet, physical activity and family history of cardiovascular disease.
Results: The median age was 36.9 years, and 30 years at transplant. The prevalence rate of MS was similar among transplant recipients (22.1%) and controls (20.8%). However, the prevalence of IR was significantly higher among patients (20%) than controls (6.5%). The most frequent components were arterial hypertension (36.2%) and central obesity (32.7%). MS was more prevalent among women older than 30 years at transplant. MS diagnosis before HSCT and obesity were significantly associated with MS and IR. However, hypogonadism was significantly related only with MS. Hormone therapy decreased by 75% the risk of MS, but the risk reduction for IR was not significant.
Conclusions: The prevalence of IR was significantly higher among HSCT recipients. MS diagnosis before HSCT, obesity, hyperinsulinemia and hypogonadism were independent predictors of MS. Only obesity and MS diagnosis before HSCT were independent predictors of IR. Further studies will be necessary to define the best hormone therapy for prevention of these metabolic disorders in HSCT recipients.

P663
Excess of veno-occlusive disease in the experimental arm of a clinical trial studying the effect of maintaining a higher haemoglobin level on neutropenia duration after bone marrow transplantation
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Introduction: Children receiving a myeloablative regimen for their bone marrow transplantation (BMT) will experience severe neutropenia. Studies conducted in the '70s and '80s suggested that if the hemoglobin (Hb) level could be kept at a higher level, the neutrophil recovery would be accelerated. The aim of this clinical trial was to assess whether maintaining a higher Hb level after allogeneic BMT would accelerate neutrophil recovery.
Methods: This clinical trial was a multicenter randomized controlled study involving all canadian pediatric transplant centers (NCT00937053). Children aged from 1 to 18 years who were undergoing an allogeneic BMT for a malignant or benign disease (except sickle cell disease) were randomised between standard transfusion strategy (control arm) and higher trigger transfusion strategy (experimental arm). Patients in the experimental group were transfused with packed red blood cells (pRBCs) to maintain

an Hb level ≥ 120 g/L while those in the control group were transfused to maintain an Hb level ≥ 70 g/L. Conditioning regimen was myeloablative in all cases. No hematopoietic growth factor was planned before BMT.
Results: Sixty patients were expected to be randomized over a two-year period. The study was stopped prematurely by the data safety monitoring board after six patients (4 females, 2 men) were recruited (three in each arm). All patients randomised to the experimental arm experienced veno-occlusive disease (VOD) while there were none in the control arm (P=0.05 by unilateral t test). VOD characteristics for each patient are shown in the table below.
Conclusions: Maintaining a Hb level higher than 120 g/L was associated with VOD in this clinical trial. No other risk factor was identified. Increased viscosity might be an etiological factor. Further research is needed to better understand the pathophysiolology.

	Patient 1	Patient 2	Patient 3
Occurrence day	+14	+19	+10
Maximum total bilirubin (mcg/L)	30	312	866
Complications	Ascite Pleural effusion Hypoxemia	Hepatic encephalopathy Ascite Coma Portal vein thrombosis	Ascite Right pleural effusion Renal failure Portal vein thrombosis
Treatments	Supportive care	Intensive care Defibrotide Molecular Adsorbent Recycling System (MARS)	Intensive care Defibrotide Hemodialysis

P664
Predictive models of idiopathic pneumonia syndrome in allogeneic stem cell transplantation patients
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Allogeneic hematopoietic stem cell transplantation (SCT) is an important treatment strategy for number of malignant and non-malignant conditions using stem cells commonly derived from bone marrow. Complications of SCT include idiopathic pneumonia syndrome (IPS). Research in animal models of the disease suggests that IPS involves an immune mediated attack involving elements of both the adaptive and the innate immune system. However, the etiology in humans is not as well understood. To define the disease pathway in humans, we performed two separate label-free, proteomics experiments to define the plasma protein profiles of bone marrow transplant patients including those who developed IPS compared to transplant recipients who did not develop complications. First, we identified disease progression associated proteins by an analysis of their intensity changes over two time points during progression to IPS (at day 0 vs. day of diagnosis). This revealed a set of 81 progression-associated proteins, many that are associated with the innate immune system, which potentially contribute to the pathophysiology of IPS and reveal similarities between IPS in humans and animal models. In the second verification analysis, we performed label free protein expression analysis on a larger cohort of patients focusing on predictors of disease and treatment response using only samples collected at day of transplant (day 0 of study) to see if proteins we discovered changing over time and disease in the first study were effective variables for patient stratification. These results identified a set of robust plasma proteomic markers suitable for SCT patient stratification that can predict patients who are likely to develop IPS and among that group, identify those who are responders and non-responders to entanercerpt (Enbrel) therapy. As such anti-TNF-α therapies are currently being developed as treatments for a number of immune related disease conditions, these results represent the basis for personalized medicine approaches ripe for further development of companion diagnostics in the field.

P665
Palonosetron versus ondansetron as prophylaxis of chemotherapy-induced nausea and vomiting: a single-centre experience
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Introduction: Chemotherapy induced nausea and vomiting (CINV) is a major issue in the setting of hematopoietic stem cell transplantation (HSCT). Palonosetron was recently introduced for CINV prophylaxis with promising results. To address the efficacy of Palonosetron in the setting of HSCT, a randomized open study (treatment / control ratio 1:2) was conducted in our Unit, starting from January 2008.
Methods: Patients (pts) were randomized to receive CINV prophylaxis with steroids plus either Ondansetron (O-arm: 8 mg iv twice a day, days -2,-1, 0 or -1, 0, for high or intermediate dose melphalan conditioning, respectively; 4 mg twice a day iv, days -7,-6,-5,-4,-3,-2 for multiple days conditioning) or Palonosetron (P-arm: 0.25 mg iv, once a day, day -2 or -1, for high or intermediate dose melphalan conditioning, respectively; 0.25 iv days -7,-5,-3 for multiple day conditioning). Nausea and vomiting were assessed daily, from the start of conditioning regimen to day +20 or discharge and symptoms severity was measured accordingly to CTC NCI 3.0 scale. Acute nausea or vomiting onset were defined: occurrence of at least one episode of >0 grade nausea or vomiting, during administration of conditioning regimen to HSCT; delayed nausea or vomiting were defined as: presence of at least one episode of >0 grade nausea or vomiting, in the interval from HSCT (day 0) to day +5.
Results: From January 2008 to June 2010, 54 pts were randomized (38 in O-arm and 16 in P-arm). Median age was 52 (20-66), 32 were male and 22 female. Diagnosis was multiple myeloma in 26 (48%), lymphoma in 20 (37%), acute leukemia in 6 (11%), other in 2 (4%). Multiple days or single day conditioning was planned in 26 (48%) and 28 (52%) pts, respectively; transplant was autologous or allogeneic in 51 (95%) and 3 (6%) patients, respectively. Acute vomiting occurred in 16/38 (42%) and 1 /16 (6%) pts in O-arm vs P-arm, respectively (p=0,01); delayed vomiting in 19 /38 (50%) and 11 /16 (69%) pts in O-arm vs P-arm, respectively (p=NS); acute nausea occurred in 31 /38 (82%) and 9/16 (56%) pts in O-arm and P-arm, respectively (p=0,05); delayed nausea occurred in 27/38 (71%) and 14/16 (88%) pts in O-arm and P-arm, respectively (p=NS).
Conclusions: Our study suggests an advantage of Palonosetron vs Ondansetron on acute CINV prophylaxis. Larger studies are needed to better define the optimal CINV management in patients undergoing high dose chemotherapy and HSCT.

P666
Efficiency of exclusive enteral nutrition in immediate post stem cell transplantation in children
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Parenteral nutrition (PN) has been considered as the “method of choice” for nutritional support of patients undergoing stem cell transplantation (SCT) with myeloablative conditioning. Due to PN’s adverse effects (e.g., mucosal atrophy, infectious complications, and hepatic dysfunction), we have used enteral nutrition (EN) as a first feeding option after SCT. Our study has assessed the efficiency of EN after allo-SCT in a paediatric population.

We included all patients under 18 years old who underwent allo-SCT with myeloablative conditioning regimen in our center between 2003 and 2010. EN was planned to start the first day after transplantation via a nasogastric feeding tube. In cases of vomiting, patient refusal, weight loss or grade III-IV gut graft-versus-host disease (GvHD), PN was administered in addition to, or replacing, EN. Nutrition status was assessed by 'weight for height and age' Z-score and by percentage change in body weight from day 0 to discharge. Our EN program was supported by a committed multi-disciplinary team (nurses, gastroenterologist and dieticians).
Fifty-nine children underwent allo-SCT during that period. All of them received EN at day 1. We compared two groups: 44 (75%) patients who were successfully managed on EN (group 1) versus 15 patients who required PN (n=13) or lost more than 10% of weight with exclusive EN (n=2) (group 2). The mean weight loss at discharge of group 1 was 0,68% compared to 4% for group 2 (p=0,03). There was no difference in weight Z-score between the 2 groups during the hospitalisation (figure 1).
In group two, 7 out of 15 patients had EN failure for gut intolerance, 5 required PN for vomiting, or insufficient volume of EN with weight loss, 2 out of the 5 refused the replacement of the tube after 2 extrusions by vomiting. Six out-of-15 had a gut GvHD in which EN was systematically stopped and replaced by PN. Total body irradiation and oral mucositis grade ≥ III were not associated with group 2. Time of transplant (before 2006) and age over twelve were associated with group 2 (p=0,04 and 0,05).
In conclusion, EN support is efficient in children undergoing SCT in spite of gut toxicity related to the conditioning regimen. EN is a physiological way of delivering nutrition and can offer cost savings. Enteral feeding should always be considered as the first option of nutrition support for these patients and PN should be used only as a rescue.

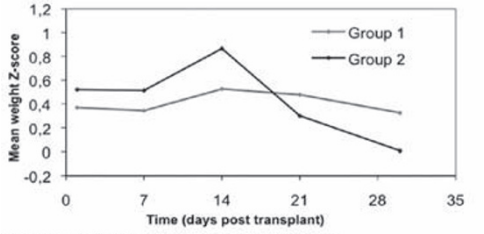

Figure 1 Mean weight Z-score during hospitalisation in the 2 groups.
Group 1: successful EN (weight loss < 10% at discharge and no parenteral nutrition).
Group 2: the others. There were no significant differences between groups at D0, 7, 14, 21 and discharge (t test).

P667
Allogeneic stem cell transplantation in elderly patients - a retrospective single-centre study
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The incidence of cancer increases with age. However, for a long time allogeneic SCT has been considered to be contraindicated in elderly patients - due to inacceptable high early treatment-related morbidity and mortality. The introduction of reduced-intensity conditioning or non-myeloablative allogeneic SCT permitted patients up to the age of 75 years to undergo allogeneic SCT. Data on long-term survival and complications of these patients, however, are still limited.
We evaluated the survival and the incidence of developing complications after SCT of adult patients transplanted for the first time with allogeneic stem cells in Innsbruck (n=371). The cohort was further divided into patients > 50 years (n=116) and patients < 50 years (n=255). The probability of overall survival was estimated using the Kaplan-Meier method and the incidence of developing relapse and acute and chronic graft-versus-host disease (GvHD) was analyzed by cumulative incidence. Statistical significance was determined using the log-rank test (NCSS statistical software package).
The overall survival six years after allogeneic SCT of elderly patients was 33% and did not significantly differ from that of younger patients (42%). Interestingly, elderly patients receiving myeloablative conditioning displayed a better overall survival (65% after 5,6 years; n=31) than elderly patients with reduced-intensity conditioning (29%; n=85; p=0.0347). Whereas the donor type (identical sibling/matched unrelated volunteer donor/mismatched either related or unrelated donor) did not have an impact on survival of younger patients, the donor type was crucial in elderly patients. Elderly patients with an identical sibling displayed a survival rate of 57% 1,4 years after SCT, with a matched unrelated donor of 41% and with a mismatched donor of 24% (p=0.0057). The cumulative incidence of developing relapse was significantly higher in elderly patients (58%; n=39) than in younger patients (48%; n=70; p=0.0393). No differences between both cohorts were detected for the development of acute and chronic GvHD.
These data provide evidence that allogeneic SCT may be a treatment option for elderly patients with an identical family or unrelated donor. However, detailed retrospective analyses and especially prospective studies are needed to substantiate the benefit of this treatment for elderly patients.

P668
Activation of macrovascular and microvascular endothelia caused by autologous haematopoietic stem cell transplantation is prevented by defibrotide
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There is endothelial activation and damage in association with autologous hematopoietic stem cell transplantation (HSCT). Through the present study, we have characterized the activation and damage of endothelial cells of both macro (HUVEC) and microvascular (HMEC) origin, occurring early after autologous HSCT, and the potential protective effect of defibrotide (DF). Sera samples from patients were collected before conditioning (Pre), at the time of transplantation (day 0), and at days 7, 14 and 21 after autologous HSCT. Changes in the expression of endothelial cell receptors at the surface, presence and reactivity of extracellular adhesive proteins, and the signaling pathways involved were analyzed. The expression of ICAM-1 at the cell surface increased progressively in both HUVEC and HMEC. However, a more prothrombotic profile characterized by an increased adhesion of platelets on the extracellular matrices generated was denoted for HMEC, in particular at the time of transplantation (day 0). These findings reflect the deleterious effect of the conditioning treatment on the endothelium, especially at a microvascular location. Interestingly, this observation correlated with a higher increase in the expression of both tissue factor and von Willebrand factor on the extracellular matrix, together with activation of intracellular p38 MAPK and Akt, and structural alterations of the endothelial cell cytoskeleton. Previous exposure and continuous incubation of cells with DF prevented the signs of activation and damage induced by the autologous sera, with stabilization of the endothelial cell cytoskeleton. These observations corroborate that conditioning treatment in autologous HSCT induces a proinflammatory and a prothrombotic phenotype, specially at a microvascular location. Moreover, our present results indicate that DF has protective antiinflammatory and antithrombotic effects in this setting.

P669

Echocardiographic findings following 10-14 Gy total body irradiation in long-term survivors of stem cell transplantation for leukaemia

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High dose radiotherapy and chemotherapy can cause irreversible myocardial damage. Doses of irradiation as low as 5Gy have been associated with cardiotoxicity and combinations of chemotherapy plus radiotherapy may be particularly toxic. In this centre we routinely use 10-14Gy TBI together with cyclophosphamide as pre-transplant conditioning for myeloablative SCT. In this study we have investigated the prevalence of echocardiographic abnormalities in consecutive patients attending a dedicated clinic for long-term survivors of transplant.
Valvular abnormalities were recorded and left ventricular ejection fractions (LVEF) calculated by Biplane Simpsons method. In addition we collected data on chronic graft-versus host disease (cGVHD), hypertension and dyslipidaemia. Data were compared to 21 normal controls matched for age and sex using the Mann-Whitney U test for non-parametric samples.
Of 50 patients (26 female), 41 were treated for CML, 9 for AML and 6 for ALL. The source of stem cells was an HLA-identical sibling in 38 patients and an unrelated donor in the remainder. The median dose of TBI was 12 Gy (range 10-14). The median follow-up was 19 y (range 8-29) and the median age at follow-up was 48 y for males (range 37-66) and 54y for females (range 33-75). The control group consisted of 10 males, median age 46y (range 36-66) and 11 females, median age 53y (range 47-69).
6/50 patients (12%) had globally reduced LVEF (<55%) on echo with mildly hypokinetic left ventricles compared to 1/21 (4.8%) in the control group. This was not statistically significant. There was no dose-response relationship between dose of TBI and LVEF and there was no significant difference in LVEF between patients who had hypertension and/or hyperlipidaemia versus those who had neither.
10/50 patients (20%) had mild or moderate valve abnormalities compared to 3/21 (14.3%) in the control group. The difference was not significant. 4 patients had mild aortic regurgitation (AR), 1 mild mitral regurgitation (MR), 2 mild pulmonary regurgitation, 1 mild AR and MR, and 2 moderate MR. There was no correlation between the prevalence of valvular abnormalities and patient age or with cGVHD.
We conclude that the prevalence of significant cardiotoxicity identified on echocardiography in this group of patients is low. Serial measurements over longer periods may be necessary identify patients in whom toxicity from chemoradiotherapy translates into significant cardiac dysfunction in later life.

P670

Outcomes after major or bidirectional ABO mismatched allogeneic haematopoietic progenitor cell transplantation following pre-transplant isoagglutinin reduction with donor type secretor plasma ± PEX

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Background: Major ABO mismatch in haematopoietic progenitor cell transplantation (HPCT) is associated with a range of immunohaematological consequences including progenitor cell infusion (PCI) related haemolysis, delayed red cell engraftment and pure red cell aplasia (PRCA). Although pre-transplant (recipient) isoagglutinin reduction may be associated with decreased immunohaematological complications in this setting, there is no consensus with respect to strategies for isoagglutinin reduction in major ABO-mismatched HSCT.
Aims: To assess the efficacy of a standardized pre-transplant isoagglutinin reduction strategy incorporating donor type secretor plasma infusions ± plasma exchange (PEX) to prevent PCI associated haemolysis and PRCA in major / bidirectional ABO mismatched peripheral blood progenitor HPCT performed at our institution.
Methods: All major/bidirectional ABO mismatched peripheral blood progenitor HPCT performed between 1999 and 2010 were identified from an institutional data base. Immunohaematological outcomes were determined retrospectively by review of individual medial records.
Results: In total 110 major/bidirectional ABO mismatched HPCT had been performed. No patient developed haemolysis post PCI. With respect to PCA incidence, 16 patients (15%) were excluded due to early mortality, and 3 (3%) due to incomplete data; of the remaining 91 patients, 5 (5%) developed PRCA. Patients with PRCA had significantly higher pre-transplant isoagglutinin titres (p=0.0001) compared to those who did not develop PRCA. There was no significant difference in isoagglutinin type (anti A vs anti B), donor recipient sex mismatch (sex matched vs sex mismatched), donor type (sibling vs unrelated) or conditioning regimen (reduced intensity conditioning vs myeloablative) between patients developing PRCA or not. 4 of 5 patients with PRCA had subsequent complete remission with reduction or withdrawal of immunosuppression alone.
Conclusions: Use of a standardized pre-transplant isoagglutinin reduction strategy including donor type secretor plasma infusions is both safe and efficient in preventing PCI associated haemolysis and is associated with low rates of post-transplant PRCA.

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Nutritional parameters as predictors of transplant outcome

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Objectives: Patients undergoing bone marrow transplant (BMT) are at high risk for malnutrition due to mucositis, vomiting and diarrhea. Decline in nutritional status is presumed to be a negative prognostic indicator for BMT outcome. The aim of the study was evaluating nutritional parameters in patients prior to and during allogeneic BMT.
Methods: 43 allogeneic BMT patients receiving total parenteral nutrition (TPN) were enrolled. 24 patients underwent an allogeneic transplant from matched related donor, 15 from matched unrelated donor and 5 haploidentical BMT. 32 patients received myeloablative conditioning, and in 12, reduced-intensity conditioning was used. Median age was 35.5 years (17–72). Nutritional status (NS) (determined by body mass index (BMI), total cholesterol (TC) and albumin (Alb) levels) was correlated with rate of infection, survival and engraftment.
Results: Nutritional status and prognosis. Admission levels: Higher TC levels were associated with a lower infection rate during hospitalization (p=0.019). Mortality rate was higher among patients with a lower NS (p=0.04). BMI<20/>30 vs. normal BMI was associated with delayed engraftment and increased mortality (NS).
During hospitalization: Lower Alb and TC levels were inversely related to the number of infections (p=0.026, p=0.006, respectively), which was directly associated with mortality. Mean Alb levels among survivors were higher compared with patients who died (p=0.001). Among patients who survived, mean TC level was 240.9±66.9 mg/dl vs. 199.28±58 mg/dl among those who succumbed (p=0.031). Alimentation Average duration of TPN feeding was 22.08±15.1 days. 59.5% of patients receiving TPN failed to fully attain the planned nutritional goal, the most common cause (40.5%) being hypertriglyceridemia. Average min. Alb in patients who did not attain their nutritional goal was lower (2.03±0.5 g/dl vs. 2.46±0.5 g/dl) than in those who did (p=0.014). Survival rate was higher in patients who achieved their nutritional goal (82.4 % vs. 56%, p=0.075).
Conclusions: Patients' nutritional status before and during transplant has a crucial impact on the risk of complications and survival. TPN remains the most successful method for feeding

patients after allogeneic transplantation, despite known complications. Therefore, individually tailored feeding plans adjusting to the changing conditions of each patient are warranted. A pre-hospitalization feeding plan might be beneficial for this patient group.

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Nationwide survey of physical exercises for allogeneic haematopoietic stem cell transplantation recipients in Japan

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Purpose: In Europe and the US, physical exercises are recommended for patients who underwent hematopoietic stem cell transplantation (HSCT) to improve motor function and quality of life. Because it is unclear whether these patients receive such care in Japan, we conducted a nationwide survey of physical exercises received by HSCT recipients.
Methods: We mailed surveys in August 2010 to 128 institutions where at least more than 4 allogeneic HSCT (not including pediatric cases) were performed in 2008. The questionnaires asked for hospital statistics, the implementation of physical exercises for HSCT recipients, and the extent of team approaches to medical care which was evaluated by a visual analogue scale (VAS). No progress was indicated by 0 mm, while 100 mm indicated major progress.
Results: Among the 77 institutions (60.2%) that replied, the ranges in numbers (median) of beds, hematology physicians, physical therapists and HSCT per year were 135–1423 (700), 2–30 (6), 0–23 (8) and 5–107 (14), respectively. The team approaches to medical care, including pharmacologic care, oral care, nutrition support, physical exercise, psychiatric care and use of a HSCT coordinator were (in mm) 70.2±25.6, 62.3±29.7, 51.7±25.4, 49.5±33.5, 45.1±32.5 and 15.8±27.8, respectively, on the VAS. Correlations between the extent of physical exercises received and numbers of physical therapists (r=0.44) and number of physical therapists per bed (r=0.41) were observed. Twenty-seven institutions (35.1%) provided physical exercises to all HSCT recipients, 13 (16.9%) gave physical exercises to those who had lower performance or activities of daily living, and 37 did not offer physical exercises at all. Twenty-four institutions (60.0%) provided physical exercises before, during and after HSCT, 3 (7.5%) gave physical exercises during and after HSCT, and 10 (25.0%) provided physical exercises only after HSCT. Physical exercises included muscle strengthen training, endurance training and stretching; however, the exercise load and programs varied.
Discussion: We determined that physical exercises for HSCT recipients were not sufficiently widespread in Japan, in part due to staffing at these institutions. We also found variations in the length and types of physical exercise programs. Therefore, we propose to conduct a multicenter study with the Japanese Society for Hematopoietic Cell Transplantation to establish evidence-based guidelines and a standard program of physical exercises for HSCT recipients.

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Glycogen phosphorylase BB as a marker of cardiac toxicity induced by high-dose and conventional chemotherapy for acute leukaemia

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Objectives: Cardiac toxicity is a potentially serious complication of anticancer therapy that can significantly impair patient's quality of life. The greatest risk for development of cardiotoxicity is represented by anthracyclines (ANT) and high-dose chemotherapy (HD-CT). The objective of our study was to assess cardiac toxicity of HD-CT followed by hematopoietic cell transplantation (HCT) and conventional CT with multiple biomarkers of cardiac injury – glycogen phosphorylase BB (GPBB), heart-type fatty acid binding protein (H-FABP), cardiac troponin I (cTnI), cardiac troponin T (cTnT), creatine kinase MB (CK-MB mass), myoglobin.
Methods: A total of 47 adult acute leukemia patients were studied – 23 patients treated with HD-CT followed by HCT and 24 patients treated with conventional CT containing ANT (cumulative dose 463.2 ± 114.3 mg/m^2). Cardiac biomarkers were measured before treatment (before HD-CT/CT), after HD-CT and after HCT in the first group; after first and last CT with ANT in the second group. Values above the reference range recommended by the manufacturers (Randox, Roche) were considered elevated. The cut-off values for cardiac injury were as follows: 7.30 µg/L for GPBB, 4.50 µg/L for H-FABP, 0.40 µg/L for cTnI, 4.80 µg/L for CK-MB mass and 76.0 µg/L for myoglobin.
Results: Before HD-CT/CT, all biomarkers were below the cut-offs. GPBB increased above the cut-off (7.30 µg/L) in 5 (21.7%) patients after HD-CT and remained elevated in 5 (21.7%) patients after HCT. GPBB increased above the cut-off in 4 (16.7 %) patients after first CT and in 5 (20.8 %) patients after last CT with ANT. CTnI became elevated (above 0.40 µg/L) in 2 (8.3%) patients after first and last CT with ANT. Both patients with cTnI positivity had elevated GPBB. Other biomarkers remained below the cut-offs.
Conclusion: Our results suggest that GPBB could become a sensitive biomarker for detection of acute cardiotoxicity associated with HD-CT followed by HCT and conventional CT containing ANT for acute leukemia. The predictive value for development of therapy-related cardiomyopathy in the future is not known and will be evaluated during a prospective follow-up. Based on our data, a larger prospective and multicenter study would be needed.
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P674

Anti-thymocyte globulin and platelet transfusions during reduced-intensity conditioning regimen

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Background: Anti-thymocyte globulin (ATG) is used as part of several conditioning regimen in order to reduce graft failure and graft versus host disease (GVHD). Acute thrombocytopenia is frequently reported, and close platelet monitoring is required. The aim of this retrospective study was to evaluate which patients required close monitoring and platelet transfusion during ATG, the incidence of bleeding events, and factors associated with transfusion.
Patients and methods: We analyzed 98 pts affected by hematological malignancies, undergoing to reduced conditioning regimen (Fludarabine 30 mg/m^2 for 3, 4 or 5 days and Busilvex® 130 mg/m^2 for 2, 3 or 4 days) with ATG (Thymoglobuline®, at dose of 5 mg/kg/day in 2 days), between January 2009 and August 2010. During ATG administration, prophylaxis with steroids and dexchlorpheniramine was applied and prophylactic platelet transfusion was performed when platelets count reached less than 50 x 10^9/L.
Results: Of 98 patients, 80 (82%) did not receive platelet transfusion, and 18 (18%) received platelet transfusion before the first and/or second ATG dose. For all patients, the median platelet count at time of RIC and before the first ATG dose were 148 x 10^9/L and 135 x 10^9/L.
Among those not transfused, the median platelet count at time of RIC, before the first ATG dose, and after the second dose were 172 x 10^9/L (35-506), 167 x 10^9/L (44-429) and 144 x

10^9/L (38- 281). Eight patients (10%) had less than 100 x 10^9/L before the RIC and first dose ATG but did not fall below platelet threshold of 50 x 10^9/L. For transfused patients, the median platelet count at time of RIC and before the first ATG dose were 43 x 10^9/L and 43 x 10^9/L. No bleeding events are reported in both groups.
In univariate analysis age, disease, and Sorror score were not associated to an enhanced risk of transfusion. As expected, the platelet count (less than 100 x 10^9/L) before the first ATG dose was significantly associated to transfusion risk (p<0.0001).
Conclusion: This retrospective analysis suggests that: i) close platelet monitoring during ATG should be reserved to patients with a platelet count less than 100 x10^9/L; ii) using a conservative platelet levels (50 x10^9/L) to perform transfusion is effective and safe when two doses of ATG are administered. This data could afford a better planning of transfusion requirements.

P675
Increased early pulmonary dysfunction in non-myeloablative haematopoietic stem cell transplant recipients conditioned with fludarabine/melphalan/alemtuzumab but not those conditioned with fludarabine/busulphan/alemtuzumab
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Introduction: Reduced Intensity Conditioning Haematopoietic stem cell transplant (RIC HSCT) enable curative treatment in elderly or co-morbid patients. Fludarabine-Melphalan-Alemtuzumab (Campath) (FMC) and Fludarabine-Busulphan-Alemtuzumab (FBC) are the commonest conditioning regimens in the UK. Deterioration in pulmonary function after myeloablative HSCT is well recognised. We describe changes in pulmonary function following Alemtuzumab-RIC HSCT.
Methods: Pulmonary function tests (PFTs) were performed pre-transplant, 6 weeks, 3 monthly in the first year post-transplant and then annually as per the NIH recommendations. FEV1, FVC, TLCO (adjusted for Hb), KCO, TLC and RV as percentage of the pre-transplant values (100%) for 42 RIC HSCT patients, (FMC=25 (19 NHL, 2 Myeloma, 2 AML, 2 CLL), FBC =17 (13 AML, 1 T-ALL, 1 Myeloma)), between 2000 and 2009 were analysed. Median follow-up was 18 months (range 3-111).
Results: Pre-transplant, the FBC group had significantly lower TLCO compared to the FMC group (p=0.04). TLCO deteriorated immediately after transplant until 15 months (73%, (39-79%)) and plateaued up to 48 months followed by a further reduction (67%, (39-79%)) associated with an increase in TLC (112% (100-127%)) and RV (145% (117-209%)) in the FMC group. FEV1 and FVC remained stable. 6/10 FMC patients at > 48 month follow-up had TLCO <80%; with increasing RV that would be consistent with GVHD of the lung.4 of these 6 had a reduction in the first 6 months suggesting an early drop may predict long term pulmonary morbidity. A significant drop (p=0.05) in the KCO was also noted in the FMC group at 1 yr post transplant. In contrast the PFT remain stable in the FBC population upto 18 months post transplant. Pre-transplant TLCO did not predict further deterioration in lung function (p=0.16), nor did donor type (siblings vs unrelated (p=0.39).
Conclusions: Thus FMC conditioning associates with a greater pulmonary morbidity post transplant which may be due to the differing diseases treated with this regime (FMC - lymphoma; FBC - acute myeloid leukaemia/myelodysplastic syndrome) or the regimen itself. Long term, pulmonary function measurements in the FMC population, particularly in those who experience a drop in TLCO in the first 6 months is recommended.

P676
Better outcome of endocrine functions in thalassaemia patients transplanted before the age of seven
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Thalassemia major (TM) patients frequently suffer from growth delay and endocrine dysfunction either because of iron overload as a consequence of repeated transfusions or because of conditioning regimen and complications like graft versus host disease as a part of hematopoetic stem cell transplantation (HSCT). In this study 41 transplanted TM patients who had been followed at least 2 years after HSCT were recruited for study and were assessed in respect to endocrine dysfunctions according to transplantation age. The median age of the patients is 12.4±5.4 with a median transplantation age that was 7 years (range 3,3-24 years). Patients were classified into two groups according to age of transplantation as being younger or older than 7 years old, 21 and 20 patients in each group, respectively. The height SDS scores were found better in patients whose HSCT age were under 7 years old compared to older than 7 (p=0,02). Significantly negative correlation was found between height SDS and HSCT age (p=0,008, r= -0,40). No relationship was seen between HSCT age and weight SDS values (p=0.56). DEXA Z scores of femur neck and L2-4 vertebra were lower in patients transplanted before the age of 7 compared to older ones (p=0,032 and p=0,035 for femur neck and spine, respectively). There were negative correlations between transplant age and DEXA Z scores of both femur neck and spine (p=0.032, r = -0,34 and p=0.0001, r = -0,54, respectively). Insulin resistance was established in 12 patients. There was a positive correlation between transplant age and HOMA-IR values indicating an increased risk of the insulin resistance with increased BMT age (p=0,01, r=0.40). Hypotiroidi was documented in 4 patients out of 41 and any correlation with transplant age was not identified (p=0.53).
In conclusion, thalassemia patients transplanted younger than the age of 7 seems to have better outcome in respect to growth delay, insulin resistance and decreased BMD and it is important to recommend transplantation before the endocrine complications have not been developed yet.

P677
Physical evaluation indicates reduction of functional performance after haematopoietic stem cell transplantation
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Objective: To evaluate physical and functional performance of pts pre and post HSCT using a functional assessment.
Methods: From November 2008 to September 2010, 50 pts, median age 48 (18-67), 29 (58%) female, were enrolled in the study. Physical evaluation and data collection were performed pre, at discharge (AD), +90 and +120 after HSCT. Functional performance 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 hand strength evaluation, Schober Test (ST) for spine mobility testing and maximum and adapted activity score (MAS & AAS) of Human Activity Profile (HAP) questionnaire for function on daily activities evaluation.
Results: 50 pts with hematological malignancies were evaluated pre HSCT, but 3 died, 2 refused and 1 was excluded. 44/50 (88%) underwent HSCT, 21 allogeneic and 23 autologous; 32/44 (72%) pts performed pre and AD evaluations; 12 did not perform AD evaluation: 9 died prematurely and 3 did not discharge yet. 20/32 (62%) pts performed +90 evaluation and 12 pts did not: 7 did not achieve time to evaluate, 3 died prematurely, 1 relapsed and 1 refused; 13/20 (65%) pts performed +120 evaluation; 3 died, 3 without enough time and 1 refused. Among groups who performed pre and post evaluations, we found significant

lower values in the AD evaluation: 2MWT (p=0.007), GS for right and left hand (p=0.004 & 0.007), ST (p<0.0001), MAS and AAS (p<0.0001); and higher values in HR (p= 0.02). At +90, GS for right and left hand maintained lower (p=0.006 & 0.02) and at +120 AAS declined again (p=0.004). AD results indicate decrease on functional performance. At +90 pts maintain deficits just on GS for both hands, indicating poor recovery for strength and +120 recovers all physical capacity, apparently.
Conclusion: Our results showed there were significant physical losses AD, nevertheless, on +90 and +120 pts seem to recover in most of parameters analyzed. These results may guide preventive measures and conduct a better rehabilitation program on post HSCT period.
Support: FAPESP.

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A possible role of recombinant thrombomodulin for a treatment of sinusoidal obstruction syndrome

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Introduction: Sinusoidal obstruction syndrome (SOS) remains one of the most serious complications after allogeneic hematopoietic stem cell transplantaion (allo-HSCT). Successful treatment of SOS with defibrotide (DF) or prostaglandin E1 (PGE1) was reported, but the efficacy remains controversial.
Patients and methods: To elucidate the efficacy of recombinant human soluble thrombomodulin (rTM) for SOS, which has recently been approved for treatment of DIC in Japan, we analyzed the data of patients who were underwent allo-HSCT at Fukuoka Bone Marrow Transplantation Group (FBMTG) from January 2008 to December 2009. Out of 229 patients, 13 were diagnosed as SOS despite under the prevention with ursodeoxycholic acid and heparin.
Results: Out of 13 pts, 4 patients were treated with PGE1 (PGE1 group), 4 were treated with DF (DF group), and 5 with rTM (380 U/kg/day, rTM group). Interestingly, the symptom disappeared more quickly in patients with rTM group than PGE1 and DF group, especially regarding decrease of body weight. Furthermore, patients in rTM group tended to develop less subsequent acute graft-versus-host disease (GVHD) after recovery from SOS than in DF group (Table 1A and B).
Discussion: These results indicate that rTM could possess not only the ability of anticoagulation but also antiinflammation, which has recently been reported about the relationship between acute GVHD and damage associated molecular patterns (DAMPs). Together, these results suggest that rTM could be considered as a treatment option for SOS. Further study is required to determine a role of rTM in GVHD besides SOS.

Table 1A.

Pt	Disease	Age	Gender	Stem cell	Times of transplantation	Disease status at transplantation	Conditioning	Treatment for SOS
1	NHL (SLL)	48	F	URBM	1st	Non CR	TBI/CY	rTM
2	ALL (T-LBL)	29	M	UCB	2nd	Non CR	Flu/Mel/TBI	rTM
3	AML/MDS	63	M	RPB	1st	Non CR	Flu/BU16	rTM + PGE1
4	ALL	52	F	URBM	1st	CR	TBI/CY	rTM + PGE1
5	MDS (RAEB)	36	M	UCB	2nd	Rejection	Flu/Mel/TBI	rTM + DF
6	AML (M4)	31	M	UCB	3rd	Non CR	Flu/Mel/TBI	PGE1
7	MF	49	M	URBM	1st	Non CR	Flu/BU8/TBI	PGE1
8	AML (M4)	31	M	UCB	2nd	Non CR	Flu/Mel/TBI	PGE1
9	AML/MDS	56	M	RPB	1st	Non CR	Flu/BU16	PGE1
10	ATL	60	F	URBM	1st	Non CR	TBI/CY	DF
11	ALL (Ph positive)	55	M	URBM	1st	CR	TBI/CY	DF
12	NHL (DLBL)	53	M	URBM	1st	Non CR	TBI/CY	DF
13	NHL (FL)	57	F	UCB	1st	Non CR	Flu/Mel/TBI	DF + PGE1

Table 1B.

Pt	Disease	Treatment for SOS	Alive/ Dead	Survival (days)	Disease status after transplantation	CHDF	Acute GVHD	Cause of death
1	NHL (SLL)	rTM	Alive	205	CR		II	
2	ALL (T-LBL)	rTM	Alive	373	CR		0	
3	AML/MDS	rTM + PGE1	Dead	14	Non CR		NE	Disease
4	ALL	rTM + PGE1	Dead	26	CR	Yes	0	SOS
5	MDS (RAEB)	rTM + DF	Dead	44	CR		0	Infection
6	AML (M4)	PGE1	Dead	392	Non CR		II	Disease
7	MF	PGE1	Alive	384	CR		II	
8	AML (M4)	PGE1	Dead	72	Non CR		0	Disease
9	AML/MDS	PGE1	Dead	28	Non CR		0	Disease/SOS
10	ATL	DF	Dead	33	NE		II	SOS
11	ALL (Ph positive)	DF	Dead	64	CR	Yes	II	SOS
12	NHL (DLBL)	DF	Dead	48	NE	Yes	III	SOS
13	NHL (FL)	DF + PGE1	Dead	89	CR	Yes	II	Infection

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Quality of life assessment in HSCT-performed thalassaemia major patients

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Today the most crucial issue in thalassemia major (TM) patients is to provide a better quality of life that is worsened by hospital visits, iron chelation and economical burden. HSCT remains the only proven curative therapy for transfusion-dependent thalassemia at present, although there is no comprehensive study for its effect on quality of life (QoL).
We have studied the QoL effect of HSCT in 50 non-transplanted and 49 transplanted patients who were transplanted at least two years before. Two health related quality of life (HRQoL) instruments validated for Turkish people were used in our study. PedsQL questionnaire was used for the patients under 18 years old as a self report and for their parents as a proxy report. The instrument was divided to subgroups for 2-4, 5-7, 8-12 and 13-18 years old patients as the instrument offered. Two to four years old subgroup questionnaire was administered only to parents. For patients above 18 years old World Health Organization's WHOQoL-BREF questionnaire was used. All the questionnaires were performed with the assistance of study coordinators.
Our study demonstrated that QoL in HSCT performed patients is not inferior than the transfusion dependent group and even higher in some areas. Higher QoL was determined in HSCT performed group who were surveyed in 5-18 years old (p=0.009). Detailed analysis marked the profound difference in 8-12 years old subgroup, particularly in physical activity questionnaires (p=0.01). QoL scores in HSCT performed adult group are higher than the transfusion dependent group, especially in physical activity domain. Transplanted adult patients rated their overall health significantly better than patients on conventional therapy but overall perception of QoL was not much differ between these groups. It was determined that class I and II mean overall QoL scores were similar. The patients who still have chronic GVHD rated worse compared to those without it.
In conclusion, TM patients who were performed HSCT at least 2 years before are not inferior than the transfusion dependent group regarding QoL and have better QoL than transfusion dependent patients in some areas. GVHD reduces the QoL significantly and it is obvious that GVHD prevention should be one of the primary goals of post HSCT follow up. QoL score is better in school children and adolescents, therefore we suggest HSCT before the primary school.

P680

Treosulphan as an effective and safe chemotherapeutic drug in preparative regimen for allogeneic haematopoietic stem cell transplantation. A paediatric single-centre experience

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Objectives: Toxicity associated with conditioning regimen (CR) given before allogeneic hematopoietic stem cell transplantation

(allo-HSCT) may increase transplant-related mortality (TRM) and morbidity. Treosulphan (T) is an alkylanting agent that has substituted busulphan in many CRs. Aims of this communication are to report the experience with T in a heterogeneous group of children and to describe extramedullary-toxicity(according to WHO score), acute and chronic Graft-versus-host disease (GvHD), and TRM.
Methods: At G.Gaslini Research Institute in Genoa–Italy between November 2007 and April 2010, 18 children received T (14 g/m^2 at -6,-5,-4), Thiotepa (8 mg/kg at -7), and Fludarabin (40 mg/m^2 at -6,-5,-4,-3) before allo-HSCT (7 pts from related and 11 from unrelated donor). 8 pts had malignancies (5 acute lymphoblastic leukemia, 2 not Hodgkin lymphoma,1 juvenile myelomonocytic leukemia) and 10 pts had not malignant diseases (2 hemophagocytic lymphohistiocytosis, 2 thalassaemia major, 3 mucopolysaccharidosis-1, 1 dyserythropoietic anemia,1 drepanocytosis, 1 congenital immunodeficiency). Source of stem cells was bone marrow in 13, peripheral blood in 3 and cord blood in 2 pts.7 pts received T before 2nd HSCT (4 rejections and 3 relapses). Median follow-up was 15 months.
Results: Median time of engraftment was 19 days (range 10-43) for nucleated cells and 24 days (14-198) for platelets.Oral mucositis occurred in 83% pts (10 pts grade 1, and 5 grade 2). 50% pts developed skin toxicity (5 pts grade 1; 2 grade 2; 2 grade 3). 44% pts developed gastrointestinal toxicity (3 pts grade 1, 4 grade 2, 1 grade 3). None developed veno-occlusive disease and 6 pts had hepatic toxicity (3 pts grade 1, 2 grade 3, 1 grade 4).Hemorrhagic cystitis appeared in 1 pt. Pulmonary toxicity occurred in 1 pt (5%) affected by capillary leak syndrome. 1 developed neurotoxicity represented by stroke-like event probably related to cerebral vasculitis. 9 pts (50%) had acute GvHD (grade ≥2 in 7), and 3 classic chronic GvHD. Among 8 pts with malignancies 4 relapsed (1 in the first 100 days, 3 in the first year), and 3 of them died. None pts died within the first 100 days after HSCT.
Conclusions: In our experience T appears to be a promising chemotherapy given before allo-HSCT in many different diseases and in 2nd HSCT to reduce transplant related toxicity. The low acute extramedullary toxicity of T allows us to use this drug in pts with a poor performance status and/or organ dysfunction who undergo a 2nd HSCT.

P681
Impact of enteral feeding in patients undergoing allogeneic haematopoietic stem cell transplantation
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Background: Allo-HSCT procedure is associated with a frequent and potentially severe malnutrition which could highly participate to the transplant-related morbidity. Optimal nutritional management is still poorly known while both enteral nutrition (EN) and parenteral nutrition (PN) are effective. We propose to evaluate the impact of EN vs PN as nutritional support in allo-HSCT.
Material and methods: We retrospectively analyzed 51 patients who needed a nutritional support after a first allo-HSCT in our center from January 2009 to September 2010. Patients with progressive disease at transplant were excluded. Fifteen patients received a myeloablative conditioning regimen and 35 a reduced intensity one. Datas were compared in a intent to treat analysis according the EN or PN initial nutritional support strategy.
Results: A total of 24 agreed to receive EN via a nasogastric feeding tube and the remaining 27 received PN. In EN group, 10/24 patients needed parenteral supplementation because of intolerance of EN. In the PN group, 3/27 patients needed enteral supplementation. No significant difference in terms of age, conditioning regimens, stem cell source, donor compatibility and CMV risk could be observed between EN and PN groups. Median follow-up was 13 months in the PN group and 6.4 months in the EN group (p=0.026). Median neutropenia and thrombopenia duration and median transfusion requirements were not significantly different. Eleven patients in EN group and 17 in PN group presented a grade 4 oral mucositis (p=NS). Incidence of bacteriemia was also not different. Interestingly, we observed a lower median length of intravenous antifungal use (0 day [0-99] in EN vs 5 days [0-93] in PN; p=0.026) and a lower rate of curative antiviral treatment requirement in the EN group (1/24 in EN vs 7/27 in PN, p=0.081). There was moreover a lower rate of replacement of central veinous catheter in EN group (3/24 in EN vs 9/27 in PN; p=0.08). Grade II-IV GVHD incidence was comparable in both groups (11/24 in EN and 15/27 in PN; p=NS). Finally, we observed a trend for a lower rate of transfer to ICU in the EN group (2/24 in EN vs 8/27 in PN; p=0.12) but early death rate (<100 days) was the same in each group (4/24 vs 4/27, p=NS).
Conclusion: EN does not influence the hematopoietic toxicity but appears to directly decrease the infectious risk in allo-HSCT. Based on these results, we are now conducting a prospective randomized trial to confirm EN benefice in allo-HSCT.

P682
Nutritional status deteriorates during allogeneic haematopoetic cell transplantation
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Malnutrition is a negative predictor for more complications, unfavourable outcome and extended length of hospital stay. We therefore performed a prospective study to answer the question: Does the alloHCT procedure deteriorate the nutritional status of the patients till day +100?
One-hundred-seven consecutive pts., median age 56 y, with mainly myeloid malignancies (82%) and advanced disease (67%) were transplanted after RIC (92%) in 77% from an URD. Body weight (BW), body mass index (BMI, kg/m²); subjective global assessment (SGA, a score for malnutrion) and the body composition (Bio Impedance Analysis, BIA) were measured before alloHCT, day +30 and day +100. BIA includes the fat mass (FM), the lean body mass (LBM) and the phase angle (PA), an important measure for malnutrition. All pts. received regularly nutrition consulting and in case of decreased calorie intake oral supplements or parenteral nutrition (65%).
Results: At admission only 26% of the pts. were moderately or severely malnourished (SGA B&C); this deteriorated extreme till day +30 (74% SGA B&C) and improved slightly until day +100 (54% SGA B&C). In all pts. BW/BMI decreased significantly from admission over day +30 to day +100 ($p < 0.0001$); but more important is the difference between the significant loss of lean body mass/m² ($p< 0.0001$) compared to fat mass/m² ($p=0.023$) in this time period. The decreased muscle mass is further documented in the significant worsening of the phase angle between admission and +30/ +100 ($p < 0.0001$). The main changes in body composition occurred between start conditioning and day +30.
Conclusion: Especially in the aplastic phase of alloHCT the nutritional status of the patients is dramatically worsening and leading to reduced muscle mass. Individual intensive nutritional support with high protein supplementation and physical exercise may stop and reverse this development. HCT outcome data (GVHD / infection / survival incidence) compared to the nutritional status will be presented.

P683
Assessment of symptom burden in patients undergoing autologous stem cell transplantation
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Patients who undergo autologous peripheral blood stem cell (PBSC) transplantation experience multiple symptoms that

affect quality of life. We assessed symptoms during the first 30 days during and after autologous PBSC transplantation to determine the severity of individual symptoms and to determine overall symptom profiles in 120 patients with lymphoid malignancies that underwent autologous transplantation in our center. Eligible patients were at least 18 years of age, spoke maternal language, and could see and hear well enough to complete the assessment measures. The assessment of symptoms was measured according to the MD Anderson recommendations of 14 symptom profiling, as well as correlated with the patient's laboratory findings, ECOG score and the profile of mood states (POMS). We retrospectively evaluated if hematopoietic cell transplantation comorbidity index (HCT-CI), karnofsky performance status (PS) and other readily available pretransplant variables concerning pretransplant mobilization strategies that can also predict the outcome of autologous recipients in our transplant center. HCT-CI risk was low in 10 (12%), intermediate in 22 (27%) high in 45 (55%) and undetermined in 5 (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. The repeated measures ANOVA for symptom severity scores (P<0.001) and symptom interference scores (P<0.001) showed only a main effect for time. None of the potential covariates (demographics, mood, quality of life, cancer diagnosis, treatment-related variables and laboratory measures) were significant. Fatigue severity revealed a significant time-by-cancer-diagnosis interaction (P=0.048), as well as pain severity (P=0.008). Sleep disturbance and lack of appetite revealed a significant time interaction (P=0.02). The symptom patterns over time demonstrated by patients with non-Hodgkin's lymphoma differed from those shown by patients with multiple myeloma. Future research can also identify differing clusters of symptoms in subgroups of patients who undergo stem cell transplantation.

P684

Life satisfaction in survivors of childhood malignant and non-malignant diseases ten years after haematopoietic stem cell transplantation does not show significant impairment compared to healthy controls: a case-matched study

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Introduction: Patients undergoing HSCT should be investigated for late physical deterioration and psychological stress which could impair quality of life. This study focused on life satisfaction (LS) in long-term survivors at least 10 years after HSCT.
Materials and methods: From March to December 2008, 55 pts (39 males, median age 25 yrs), who underwent to allogeneic (49) or autologous (6) HSCT for childhood malignant (52) and non-malignant (3) diseases at least 10 years before the study, were consecutively enrolled. A control group of 98 young adults (59 males, median age 24 yrs) was considered. A questionnaire including a modified Satisfaction with Life Domain Scale for HSCT was administered both to the pts and to the controls, after obtaining an informed consent.
Five domains (education, employment, leisure time, social relationship and perception of physical status), each containing 2 to 10 items for a total of 30 questions, were assessed. To investigate the association between the domains and the probability of LS, we performed the logistic procedure by the method of maximum likelihood. Predictive factors of LS adjusted for age and type of HSCT (as continuous variable) were evaluated.
Results: In the univariate analysis the only significant difference between the case and the control groups was the level of education (p<0.001), but the difference is probably due the fact that some patients are still on secondary school and didn't get yet the appropriate certificate. Multivariate analysis showed that the level of LS was neither significantly correlated to socio-demographic factors (including level of education, work problems, family relationship, leisure time) nor to the HSCT status. A trend in favour of control group was represented by own body perception (p=0.062).
Conclusions: The results of the current study indicate that the pts who underwent HSCT during childhood have not a significant different LS compared to healthy controls.

P685

Perceived quality of life measures in haematopoietic stem cell transplantation clinical practice: results of prospective evaluation in 142 patients

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Object: Perceived quality of life (QoL) affects patients' evaluation of treatment outcome and their compliance to demanding therapies like Haematopoietic Stem Cell Transplantation (HSCT) whose related complications can greatly affect daily life for a long period.
Aim of this study was to evaluate factors regarding both patients' clinical status and psychological well-being potentially related to Qol perceived by patients during HSCT: a better understanding of the patient's evaluation process of his QoL can help to translate QoL measures in clinical practice planning specific intervention to promote patients' QoL, improving their satisfaction to medical treatment.
Methods: From January 2008 to October 2010, 142 patients undergoing HSCT completed questionnaires measuring level of anxiety and depression (Hospital Anxiety and Depression Scale), distress (Perception of Distress Index), quality of life (Medical Outcomes Study Short form-36) and patient's style to cope with the disease (Mental Adjustment to Cancer Scale) controlling for sex, age, stage of HSCT (pre-infusion, within 1 week, 1 month, 3 months, over 3 months), diagnosis, type of transplant, disease stage, sorror comorbity index (C.I.), blood counts and fever measured the same day the patient filled the questionnaires.
These data were analyzed by the statistical software SPSS.
Results: The results show a significant negative correlation between:
- mental and physical QoL and level of anxiety, depression, distress and helplessness coping style: the best QoL is associated with the lowest level of anxiety, depression, distress and with the lowest perception of lack of psychological resources to face with the disease;
- physical QoL and C.I. and fever: the best physical Qol is associated with the lowest level of fever and C.I. score.

No correlation between QoL and other clinical measurable variables of the disease and its treatment (type of transplant, stage of HSCT, diagnosis, disease stage) was found.
Conclusions: HSCT programs should include an assessment of both physical and psychological factors affecting perceived QoL: this intervention can help the clinician to understand how patients evaluate their conditions in order to plan shared treatment goals and undertake proper preventive and therapeutical measures. This would help patients to face with all the variables (depression, anxiety, coping style, distress) that affecting QoL can interfere with willingness to adhere to the HSCT program.

P686

Natriuretic peptides as markers of acute cardiotoxicity in children undergoing haematopoietic stem cell transplantation

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Natriuretic peptides are potentially useful for early detection of cardiotoxicity (CT). Atrial Natriuretic Peptide (ANP) is secreted

as a result of an increased left atrial pressure, N-terminal fragment of Brain Natriretic Peptide (BNP) is produced by ventricles in response to ventricular dilatation and increased wall stress.
The aim of the study is to assess the frequency and significance of elevated BNP and ANP plasma levels and their correlation to echocardiographic parameters of left ventricular function in children in early postransplant period.
Patients: 31 patients treated with HSCT were included into the study, with median age 9,6 years (0,2 to 18 years), there were 22 boys and 9 girls. 12 autologous transplantations (aHSCT) were performed, 9 children were transplanted from unrelated donor (MUD) and 10 from family donor (MSD).
Methods: The plasma levels of NT-proBNP and ANP were measured once in the control group; in transplanted patients pretransplant and every week for 3 weeks in postransplant period by enzyme immunoassay. Shortening fraction (SF) and ejection fraction (EF) were assessed by echocardiography at rest prior to HSCT and about day +30 and +100 after transplantation .
Results: Baseline echocardiographic parameters were normal in all patients included into the study. Decrease of median value of SF and EF was observed in day +30 in the analyzed groups of children (n.s), remaining within normal range (FS > 28% and EF > 55%).
In all transplanted patients NT pro-BNP plasma concentrations were significantly elevated on days -7 to +7 ($p<0,01$), ANP plasma concentrations were significantly elevated on day +7 ($p< 0,01$). This elevations in biochemical markers levels did not correlate with echocardiographical parameters evaluated on days +30 and +100 after transplantation.
According to the type of transplant NT pro-BNP and ANP plasma levels were elevated in MUD transplant recipients on days 0,+7, +14 postransplant ($p<0,05$), in MSD patients on day +7, while in aHSCT pts did not differ compared to controls.
Statistically significant correlation was found only between ANP concentration on day +14 and EF on day +100 in MUD pts ($r = -0,9$).
Conclusions:
1. Elevation of serum levels of natriuretic peptides (ANP, NT-proBNP) may indicate risk of cardiotoxicity in unrelated transplant recipients.
2. Long-term follow up is necessary to confirm the role of natriuretic peptides as markers of acute CT in selected types of transplants.

P687

Comprehensive symptom profile in patients undergoing BMT/PSCT: practicability and usefulness of the new symptom assessment tool

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Symptom severity is an important treatment outcome for patients undergoing BMT/PSCT. Recently, the new symptom assessment tool, Comprehensive Symptom Profile in Patients Undergoing BMT/PSCT (CSP-BMT), to provide comprehensive evaluation of symptoms specific for patients undergoing BMT/PSCT has been developed. We aimed to test practicability and usefulness of CSP-BMT.
114 patients who underwent BMT/PSCT were enrolled in the study. Among them there were 51 patients with hematological malignancies and 63 –with severe autoimmune disorders. Mean age was 35 years (range 18-73); male/female distribution –48/66. All the patients filled in the CSP-BMT before and at different time-points after BMT/PSCT. CSP-BMT is developed to assess the severity of 46 symptoms which occur in patients undergoing BMT/PSCT. The analysis of practicability of the CSP-BMT was conducted at the discharge.
Practicability of the CSP-BMT was shown: patients needed 10-15 min to answer it, usually without assistance; the proportion of missing values was less than 1.5% for all questions; the questionnaire found high acceptance reflected by no refusals. Usefulness of the CSP-BMT to distinguish patients in terms of severity and number of symptoms experienced was demonstrated. The information obtained was used by physicians for the decision-making. At the discharge all the patients experienced at least one symptom. 42 out of 46 tool items were registered for more than 10% patients. The majority of patients experienced fatigue (83%), skin problems (63%), hair loss while combing (55%), sleep disturbance (54%). Notably, 74 (63 %) patients experienced moderate-to-severe (7-10 on the numerical rating scale) symptoms. Among them 8 (11%) patients experienced 5 moderate-to-severe symptoms, 3 (4%) patients experienced 10 moderate-to-severe symptoms, 3 (4%) patients experienced 20 and more moderate-to-severe symptoms.
In conclusion, the CSP-BMT is a practicable and useful tool to measure symptom profile and severity in patients undergoing BMT/PSCT. Heterogeneity of patient population in terms of symptom number and severity at discharge was demonstrated. The results obtained highlight treatment related problems of patients after BMT/PSCT at discharge that need to be addressed at follow-up and should not be neglected. The data of the CSP-BMT might be of value for physicians to provide better management of this patient population.

P688

Response of steroid-resistant capillary leak syndrome to bevacizumab

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Capillary leak syndrome (CLS) is a severe complication after haematopoietic stem cell transplantation (HSCT). The underlying pathology is poorly understood, however generalized capillary endothelial cell injury in multiple organs results in a loss of intravascular fluid into interstitial spaces. Vascular endothelial growth factor (VEGF) is a potent inducer of vascular permeability and may have a crucial role in the mechanism underlying CLS formation.
Objectives: the potential role of VEGF prompted us to use the anti-VEGF antibody bevacizumab (Avastin) in patient with severe steroid-resistant CLS. Methods: a 38 years old woman with acute lymphoblastic leukemia (common B ALL) underwent sibling, ABO compatible allogeneic HSCT in first complete remission in our institution.
Results: the first CLS attack developed day 15 after HSCT (pericardial and pleural effusions, generalized edema) and responded promptly to glucocorticoids 2 mg/kg/d i.v. and appropriate supportive management. Engraftment was observed day 26 (ANC $0,5x10^9$/l and Plt $20x10^9$/l), there were no signs of graft versus host disease (GvHD). On day 84 the patient developed second attack CLS (sudden abdominal pain, decreased urine output, with pericardial and pleural effusions, ascites, hepatomegaly). Restoring glucocorticoid therapy improved CLS. Another CLS attack appeared day 153 after HSCT with chest pain, shortness of breath, ascites and peripheral edema. CT scan documented pericardial and pleural effusions, hepatomegaly and moderate ascites. This time glucocorticoids (2 mg/kg/d i.v.) and conventional supportive measures (diuretics, oral fluids restriction, nutritional support, albumin administration, etc.) were not effective and patient had to undergo repeated (3x600 ml) thoracenteses for pleural effusions. Intravenous bevacizumab (Avastin, 5 mg/kg body weight) was administered over a 90-minute period on day 174 and day 188 after HSCT. Bevacizumab infusion was well tolerated, all CLS symptoms

were ameliorated and a marked decrease in the amounts of pleural effusion was evident on the chest X-ray. On the fourth day after second bevacizumab administration the patient discharged from the hospital without any signs of CLS.
Conclusion: CLS treatment is based on systemic corticosteroids. However, in steroid-resistant CLS using bevacizumab may offer a new therapeutic strategy to counteract this severe vascular permeability disorder, especially in patients with life-threatening attacks.

P689
Late effects after haematopoietic stem cell transplantation in children with haematological malignancies diagnosed within the first year of life
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The burden of late effects after oncological treatment and/or hematopoietic stem cell transplantation (SCT) in childhood has been described, but it is still unclear if younger age at treatment is a risk factor for a higher burden of late effects.
Objective: to assess number and severity ("burden") of late effects in children at least 2 years after SCT for hematological malignancy diagnosed in the first year of life.
Patients and methods: all children diagnosed with hematological malignancy within the first year of life who underwent allogeneic SCT in our hospital from 1981 to 2006 were selected for this study. Patients who were alive at least 2 years after SCT were assessed for late effects and graded for severity of late effects according to the CTCAE vs 3.0. Unfortunately, neurocognitive and psychological late effects could not be assessed, since patients had not been seen by a psychologist.
Results: 23 patients were included in the study, transplanted at a median age of 15 months (range 6 to 57 months), for AML (n=5), ALL (n=8), CML (n=1), MDS (n=2) and JMML (n=7) diagnosed before the age of one year. Thirteen received a transplant from an identical related donor (IRD), 8 from a matched unrelated donor (MUD) and 2 from a haplo-identical parent. Stem cell source was bone marrow in 21 patients, peripheral blood stem cells in 2 (MUD) and cord blood (IRD) in one. Nine patients died, 6 due to relapse after SCT, 2 due to transplant related mortality (VOD and adenovirus infection) and one due to a traffic accident. In 14 patients, alive at least 2 years after SCT, late effects were assessed. Median age at the time of assessment of late effects was 11 years (range 2 to 27 years). Treatment received and late effects, scored per organ or as a general late effect, will be shown.
Conclusions: Eleven of 14 patients (79%) had one or more late effects. Late effects were mild or moderate (grade 1 or 2) in 10 patients and severe (grade 3) in only one patient (gonadal insufficiency). No cardiomyopathy, renal dysfunction, hearing problems or secondary malignancies were seen in these relatively young SCT survivors.
Although more late effects may develop with time, the burden of late effects in these patients, who were transplanted at a very young age (with three patients receiving TBI), appears not to be different from what has been described in older children.

P690
Long-term effect of kidney shielding during total body irradiation: a single-centre experience
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Long-term follow-up of patients undergoing allogeneic hematopoietic stem-cell transplantation (HSCT) revealed a high incidence of chronic kidney disease (CKD), prompting us to introduce kidney shielding during total body irradiation (TBI) (kidney dose of 10 Gy). However, this shielding may increase the possibility of relapse because it reduces the strength of the preparative treatment for leukemia. To evaluate the effect of the shielding maneuver, we evaluated patients with acute lymphoblastic leukemia in first complete remission who had received their first transplantation more than 5 years previously. All patients had the same preparative regimen with the exception of kidney shielding, which started in March 1999.
Fifty-five patients were included, 21 of them without shielding (GNS) and 34 with shielding (GS). The historical comparison led to more high-risk patients in the GS, considering relapse and graft-versus-host disease.
The incidence of relapse demonstrated no significant difference between the two groups (5/21 patients in the GNS and 11/34 in the GS), irrespective of Philadelphia chromosomal status. There were no cases of relapse that started in the kidney or surrounding tissue.
The incidence of acute kidney injury diagnosed according to the RIFLE criteria was very similar in the two groups whereas the incidence of CKD at five years after transplantation was significantly lower in the GS (p=0.023).
We conclude that protective shielding during TBI in the preparative regimen can suppress the incidence of CKD at 5 years after transplantation, without affecting the risk of relapse.

P691
Dysphagia profile changes according to conditioning regimens in cancer patients who underwent high-dose chemotherapy and stem cell transplantation
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Purpose: Patients that underwent high dose chemotherapy (HDC) and autologous peripheral stem cell transplantation (APSCT) or allogeneic bone marrow transplantation (ABMT) experience dysphagia frequently. This study was done to evaluate the frequency and severity of dysphagia in the early period of transplantation and the relation of conditioning regimens with dysphagia.

[P689]

Treatment and late effects in children transplanted for infant leukemia and alive more than 2 years after HSCT.

Patient	Chemo	Conditioning	Late effects											
no	Pre-HSCT	regimen	Endocrine	Cardiac	Pulmonary	Renal	Liver	Ocular	Hearing	Dental	Skin	Fatigue	Obesity	SM	No. LE
1	D, E	Cy, TDI 50y	T0, G1, Gn0, B0	n.a.	0	0	0	1	n.a.	n.a.	0	0	0	0	2
2	A, D, E	Cy, ARA-C	T1, G1, Gn3, B0	n.a.	n.a	0	0	0	n.a.	n.a	0	0	0	0	3
3*	A, D	Cy, E, TBI 70y	T2, G2, Gn n.a., B2	0	2	0	0	0	0	1	0	1	0	0	6
4	A, D, E	Bu, Cy	T0, G0, Gn n.a., B0	n.a.	1	n.a.	0	0	n.a.	n.a.	n.a.	0	2	n.a.	2
5	D, E	Bu, Cy, E, ARA-C	T0, G0, Gn1, B0	0	0	0	0	0	0	1	1	0	0	0	3
6		Bu, Cy, ARA-C	T0, G0, Gn n.a., B0	0	n.a	0	n.a.	0	0	n.a.	0	0	0	0	0
7*		Bu, Cy	T0, G0, Gn 1., B0	0	1	0	0	0	0	0	0	0	0	0	1
8*		Bu, Cy, Mel	T0, G1, Gn n.a., B0	0	1	0	0	0	0	1	0	0	0	0	3
9*	A, D, E	Cy, E, TDI 70y	T1, G2, Gn n.a., B0	0	0	0	0	0	0	0	0	0	0	0	2
10*		Bu, Cy, Mel	T0, G0, Gn n.a., B0	0	1	0	1	0	0	1	0	0	0	0	2
11*		Bu, Cy, Mel	T0, G1, Gn n.a., B0	0	n.a	0	0	0	0	2	0	0	0	0	1
12*	A, D	Bu, Cy, E	T0, G0, Gn n.a., B0	0	n.a	0	0	0	0	0	0	0	0	0	0
13*		Bu, Cy, Mel	T0, G0, Gn n.a., B0	0	n.a	0	0	0	0	0	0	0	0	0	0
14*	A, D	Bu, Cy, E	T0, G0, Gn n.a., B0	0	n.a	0	0	0	0	1	0	0	0	0	1

*: follow-up late effects clinic LUMC; Chemo before HSCT: A = alkylating drugs, D = anthracyclines like doxorubicin and daunorubicin, E = etoposide; Conditioning regimen: Cy: cyclophosphamide, TBI: total body irradiation, ARA-C: cytarabine, E: etoposide, Bu: busulphan, Mel: melfalan; Endocrine late effects: T: thyroid, G: growth/growth hormone, Gn: gonadal/infertility, B: bone (see text); n.a. = not available (relevant diagnostic test not performed or no data on presence/absence of symptoms); SM: secondary malignancy, No. LE: number of "organ systems" involved in late effects

Patients and methods: Patients with haematologic or solid tumors who underwent APSCT or ABMT were asked to score dysphagia severity daily from the first day to the tenth day of reinfusion. Scoring was performed according to a five-grade scale (0: no symptom; 1: mild; 2: moderate; 3: severe; 4: very severe). Total dysphagia score (TDS) was defined as the addition of symptom severities of dysphagia in 10 days. A total of 113 (99 APSCT and 14 ABMT) patients, 81 men (72%) and 32 women (28%) were included to the study. Median age of patients was 32 (range 15-78 years). The most frequent three diagnosis were non-Hodgkin's lymphoma (30%, n=34), Hodgkin's lymphoma (19%, n=22), and Multiple Myeloma (11%, n=12). BCNU, Etoposide, Cytarabine, and Melphalan (BEAM) (n=44), Ifosfamide, Carboplatin, and Etoposide (ICE) (n=29), Melphelan 200 mg/m^2 (M200) (n=12) and total body irradiation +Cyclophosphamide (TBI+C) (n=28) were used as conditioning regimens.
Results: All of the patients experienced dysphagia at any grade. Patients who scored grade 3 and 4 dysphagia was higher on day 7 when compared with day 1 (38.6% vs 9%, p<0.05). TDS in M200 and TBI+C group was significantly higher in days 7 to 10 when compared to BEAM and ICE groups (p<0.05). TDS was not different when compared BEAM and ICE groups. TDS was higher in women from days 7 to 10, and inverse correlation with white blood cell count was found with dysphagia in days 5, 8, 9, and 10 (p<0.05). The mean percentages of patients who scored severe or very severe dysphagia in 10 days was 13.85% in BEAM, 18.23% in ICE, 28.32% in M200 and 27.01% in TBI+C treated groups.
Conclusion: All patients that underwent HDC faced dysphagia. The ICE conditioning regimen caused dysphagia earlier, TBI+C and M200 treated patients faced more dysphagia 7 to 10 days after stem cell reinfusion.

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Second cancers and outcome after haematopoietic stem cell transplantation: long-term follow-up (1984-2008) report of a single centre

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Background: Second cancers after Hematopoietic Stem Cell Transplantation (HSCT) include posttransplant lymphoproliferative disorder (PTLD), hematologic malignancies and solid cancers.
Patients and methods: The aim of this study was to investigate second cancers and outcome in patients who underwent autologous and allogeneic HSCT. We analyzed data on 695 consecutive patients with autologous (n=474) and allogeneic (n=221) transplant at a single center between 1984-2008. The cumulative risk of developing a second cancer was calculated by Kalbfleisch and Prentice's method.
Results: The median duration of follow-up was 34 months (range, 12-185). Fifteen-year overall survival rates in patients who underwent autologous and allogeneic HSCT were 32.9% and 49.7%, respectively. In a total of 11 second cancers developed among 695 patients. The cumulative incidence of developing a second cancer at 5 and 15 years was 3.1% and 10.9%, respectively. Second cancers were hematologic malignancies in 7 patients (AML: 3, NHL: 3, PTLD: 1) and solid cancers in 4 patients (lung cancer: 2, breast cancer: 1, brain tumor: 1). Of 11 patients, seven of them died as a result of progression of the second cancer, and four patients are still alive.
Conclusions: The incidence of second cancers continues to rise without a plateau with increasing follow-up of HSCT survivors. Appropriate screening recommendations should be followed to detect and treat these cancers at an early stage.

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Early or delayed administration of G-CSF after peripheral blood stem cell transplantation in lymphoma patients

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Background: Granulocyte colony-stimulating factor (G-CSF) significantly accelerates granulocyte recovery after autologous peripheral blood stem cell transplantation (PBSCT). The optimum timing of G-CSF initiation after PBSCT is not clearly established.
Purpose: To compare time to hematologic recovery and infection rate in patients starting G-CSF on day +1 and day +5 post-transplant.
Methods: We retrospectively analyzed outcome of 125 consecutive patients treated for lymphoma in two separate hospitals according to the current local standards with G-CSF started either on day +1 after PBSCT (group G1: n=69) or on day +5 (group G5: n=56). Time to neutrophil and platelet recovery, incidence of infection, and length of stay in hospital were evaluated.
Results: The median time to absolute neutrophil count (ANC) > 0.5 x 10^9/l was 10 vs. 11 days in G1 vs. G5 group, respectively (p=0.0005). The median time to ANC > 1.0 x 10^9/l was 10 vs. 11 days in G1 vs. G5 group, respectively (p=0.08). Median time to platelet transfusion independence was 11 vs. 12 days in G1 vs. G5 group, respectively (p=0.02). There was no difference in number of infections between both groups of patients. The median duration of stay in hospital post-PBSCT was 14 vs. 15 days in the G1 vs. G5 group, respectively (p=0.01).
Conclusions: These results indicate that hematologic recovery was (median) 1-day faster and stay in hospital was 1-day shorter with G-CSF administration initiated on day +1 compared to day +5 post-PBSCT without influence on the infection incidence.

P694

Renal failure in patients treated with cisplatin prior to autologous stem cell transplantation

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Background: The combination of cisplatin, cytarabine and dexamethasone (DHAP) has proven to be an effective regimen for patients with relapsed or refractory lymphoma. However, nephrotoxicity is the major potentially limiting factor of DHAP. In fact, progressive and partially irreversible declines in renal function may develop after each successive treatment course. On the other hand, it is well known that renal failure (RF) reduces the patient survival after hematopoietic stem cell transplantation (SCT).
Objective: The aim of this study is to analyze the RF incidence and the influence of previous DHAP treatment on it, in patients undergoing autologous SCT.
Patients and method: We analyzed 39 lymphoma patients who underwent autologous SCT after conditioning with BEAM. Serum creatinine was measured on a daily basis from admission to discharge. Creatinine clearance (CrCl) was calculated according to the next formula: Cl cr (ml/min)=[(140-age) x height]/[72 x creatinine]; women correction: x 0,85. RF was defined as a >25% decrease in CrCl. Total cumulative cisplatin dose (mg/m^2) administered prior to transplant was calculated for each patient.
Results: Eighteen out of the 39 patients (46 %) had received DHAP prior to the SCT (1 course in 5 pts, 2 courses in 9 pts, and 3 courses in 4 pts). Seven patients (17,6%) developed RF. There was association between having received DHAP and the development of RF (p=0,02). In addition, a correlation was found between the decrease of CrCl and the dose of cisplatin received (r2=0,11; p=0,03). No patients required dialysis, but RF was permanent after discharge in 3 cases.

Discussion: The administration of prior DHAP was associated with the development of renal failure during the autologous SCT. Nephrotoxicity often occurred with doses lower than recommend as maximum doses. Several measures, including the avoidance of high cumulative cisplatin doses or the use of alternative regimens, as ICE or IFE, can help to reduce the incidence of RF in patients with lymphoma undergoing auto-SCT.

	With RF (n=7)	Without RF (n=32)	P
Age	59,3	48,1	0,001
Pre-SCT DHAP	6(85,71%)	12 (37,5%)	0,02
Severe infection	7 (100%)	16 (50%)	0,01
Days of hospitalization	21,8	18,9	ns
Sex female	5 (71,4%)	13 (40%)	ns
Interval between the last dose of cisplatin and the beginning of conditioning (weeks)	15,5	16,3	ns
Amikacin	2 (28,7%)	5 (25%)	ns
Mortality at day +100	0 %	0 %	ns

P695

Cytarabine-induced fever during autologous stem cell transplantation

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Background: Drug-induced fever is a febrile response associated with the administration of a drug. Antineoplastic agents are a common cause of drug-induced fever.
Methods: We analyzed the frequency and characteristics of cytarabine-induced fever (CIF) in 11 pts with AML conditioned with BEA (cytarabine 2 g/m^2/12 h, 2 days) and 41 pts with lymphoma conditioned with BEAM (cytarabine 200 mg/m^2/12 h, 4 days). CIF was considered in non-neutropenic pts, with negative cultures, and disappearance of the fever rapidly after the drug discontinuation.
Results: CIF (including fever and febricula) was detected in 40 pts (76.9%): 10 with BEA (90%, half of them being high fever) and 30 with BEAM (73.2%, a quarter of them being high fever). Mean time onset of CIF from the beginning of the drug was 32 h (7-76 h) and mean duration of CIF was 28 h (1-96 h). Maximum temperature reached was 38.6±0,5 ºC. Maculo-papular rash was detected in 17 pts (32.7%): 6 with and 11 without CIF (p=0.28). Twenty-five blood cultures, 10 urine cultures and 4 chests X-ray were performed. Antibiotics were administered to 9 patients. The conditioning was continued in all patients.
Conclusion: CIF was a very frequent event during conditioning regimens with cytarabine (BEA, BEAM). CIF started very soon after the beginning of the drug. The absence of concomitant maculo-papular rash did not rule out CIF. Considering this results, it is important to maintain a high index of suspicion of CIF in order to reduce inappropriate and expensive tests and treatments. The implementation of guidelines for the management of CIF is also desirable.

	With RF (n=7)	Without RF (n=32)	P
Age	59,3	48,1	0,001
Pre-SCT DHAP	6(85,71%)	12 (37,5%)	0,02
Severe infection	7 (100%)	16 (50%)	0,01
Days of hospitalization	21,8	18,9	ns
Sex female	5 (71,4%)	13 (40%)	ns
Interval between the last dose of cisplatin and the beginning of conditioning (weeks)	15,5	16,3	ns
Amikacin	2 (28,7%)	5 (25%)	ns
Mortality at day +100	0 %	0 %	ns

P696

Post-transplantation (at 1 year) serum ferritin is related to long-term outcome after allogeneic stem cell transplantation

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Background: To clarify the clinical significance of iron overload after allogeneic stem cell transplantation (SCT), we retrospectively assessed the association of post-SCT serum ferritin (SF) level with outcome in patients surviving for 1 year or more after SCT.
Methods: The SF was measured at preconditioning and 1 year after transplantation in patients who underwent SCT between January 2000 and September 2009. There were 86 patients (pts), including 41 with AML, 26 with ALL, 8 with MDS, and 11 with CML. Their median age was 41 years (range: 17-61 years), with 40 males and 46 females. The disease risk at transplantation was standard risk in 55 pts and high risk in 31 pts. Myeloablative preconditioning was employed for 61 pts and reduced-intensity preconditioning was done for 25 pts. The SF level was categorized as low (< 1000 ng/ml) or high (≥ 1000 ng/ml).
Results: The median (range) SF level at pre-SCT and 1 year after SCT were 770 (7-11000) and 808 (100-18000) ng/ml, respectively. The 31 pts (36%) at pre-SCT and 37 (43%) at post-SCT showed a high SF level. Pre-SCT SF, disease risk and amount of RBC transfusion within 1 year after SCT were significantly associated with post-SCT ferritin level. On univariate analysis, factors associated with worse 5-y overall survival (OS) included high SF at post-SCT (vs low: 51 vs 84%, p=0.003), high disease risk at SCT (vs standard: 38 vs 81%, p<0.001), and higher amount (>7 units) of RBC transfusion after SCT (vs lower: 57 vs 81%, p=0.025). The 5-y non relapse mortality (NRM) was associated with high disease risk (vs standard: 40 vs 12 %, p=0.034) and the presence of chronic graft-versus-host disease (vs absence: 27 vs 5%, p=0.018). The 5-y cumulative incidence of relapse was related with high SF at post-SCT (vs low: 33 vs 17%, p=0.029) and high disease risk (vs standard: 36 vs 18%, p=0.029). Multivariate analysis showed that high disease risk (HR, 3.19; CI, 1.37-7.43; p=0.007) and high SF at post-SCT (HR, 2.52; CI, 1.00-6.31; p=0.049) were independent determinants of 5-y OS. There were no significant predictors for 5-y NRM and relapse by multivariate analyses.
Conclusion: Reassessment of iron overload at 1 year after SCT predicted long-term outcome in patients surviving for 1 year or more after SCT. Although further evaluation to identify causes of high SF after SCT is necessary, these results may help to decide which patients should be treated with iron chelating therapy among long-term survivors after SCT.

P697

Effect of Etanercept and mPSL pulse therapy for idiopathic pneumonia syndrome and diffuse alveolar haemorrhage following RI-CBT

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Background: Idiopathic pneumonia syndrome (IPS) and diffuse alveolar hemorrhage (DAH) are non-infectious pulmonary complications following hematopoietic stem cell transplantation (HSCT), their precise pathogenesis and adequate treatment modalities remain to be resolved.
Objective and Method: To assess the effect of eternercept and mPSL bolus therapy for IPS/DAH after reduced-intensity cord blood transplantation (RI-CBT), we retrospectively reviewed 8 patients with IPS/DAH following RI-CBT. Diagnosis of IPS/DAH was based on clinical criteria, which included acute onset of hypoxemia with presence of diffuse pulmonary infiltrates on CT scan.
Result: Their median-age was 60 years (range; 26-64). Seven patients received RI-CBT for leukemia and 1 for malignant lymphoma. Most patients were conditioned with Fludarabine-based regimen. All grafts were 4 of 6 HLA-matched by serology, but in 6 of those grafts were 1 to 3 of 6 HLA –matched by allele level typing. IPS/DAH was diagnosed at a median of 30 days (range; 20-52) after RI-CBT. Etanercept was administered subcutaneously at a dose of 0.4 mg/kg twice weekly. All patients were administered combined with mPSL pulse therapy. Five of 8 did not respond, while the initial effect following etanercept

and mPSL pulse therapy was observed in 3 cases, but flared up after discontinuation of etanercept. Finally, all patients died of IPS/DAH progression.
Conclusion: IPS /DAH post RI-CBT are fatal pulmonary complications. Our results suggested that etanercept and mPSL pulse have only limited efficacy as a therapy for IPS/DAH post RI-CBT. Further research is needed to characterize the pathogenesis of this syndrome and to investigate the optimal therapy and prophylaxis.

P698

Higher incidence of hypertension with intravenous busulfan compared to oral busulfan in thalassaemic children who underwent haematopoietic stem cell transplantation

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Busulfan (Bu) is an alkylating agent that is frequently used as a part of the conditioning regimen prior to hematopoietic stem cell transplantation (HSCT) in adults and children with various malignant and non-malignant diseases. Although it has been shown to be effective in both oral and intravenous (iv) form, iv Bu is mostly preferred due to the inter-individual variability in exposure after oral administration. There are conflicting results of studies comparing the toxicity of oral versus iv Bu. The aim of this study is to compare the incidence of hypertension between oral and iv Bu in thalassemic children undergoing HSCT.
Between March 2008 and August 2010 forty-six children (18 male and 28 female) with β-thalassemia major underwent allogenic HSCT from matched-sibling-donor at Akdeniz University HSCT Unit. Median age at transplantation was 93 (range 24 to 350) months. In twenty-five patients (group 1) the conditoning regimen consisted of oral Bu 16 mg/kg, cyclophosphamide (CY) 200 mg/kg and anti thymocyte globulin (ATG) 30 mg/kg. The other 21 patients (group 2) received Bu 12.8 mg/kg iv, CY 200 mg/kg and ATG 30 mg/kg. Cyclosporin A (CsA) and short methotrexate were given as GVHD prophylaxis. Patients who underwent transplant with cord blood received CsA and prednisolone. Patients' files were retrospectively analyzed for hypertension. Hypertension was defined as a systolic and/or diastolic blood pressure > 95th percentile for age, sex and gender.
During hospitalization period, hypertension was diagnosed in 4 of the 25 patients in group 1 (16 %) and 12 of the 21 patients in group 2 (57%). There was significant difference between two groups (p: 0.004). All patients needed antihypertensive therapy.
In conclusion, although this is observational study and serum Bu levels are not available, we determined that use of iv Bu as part of the conditioning regimen in thalassemic children undergoing HSCT leads to a higher incidence of hypertension than does the use of oral Bu. However, prospective randomized trials are needed to confirm this data.

P699

Endocrine, bone and nutritional 'late effects' in patients with advanced stage multiple myeloma following HSCT and other intensive treatment

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Background: Modern treatment strategies in myeloma, including haemopoietic stem cell transplantation (HSCT), have increased life expectancy, but 'late effects' of treatment and disease have not been studied systematically.
Objectives: To define and characterise the spectrum of endocrine, bone and nutritional issues in intensively treated advanced but stable myeloma post HSCT.
Methods: We recruited myeloma patients who had received HSCT as part of initial treatment, and at least one subsequent treatment for progressive disease, who were in stable plateau phase off treatment for active disease. Patients were extensively screened for endocrine, bone and nutritional parameters. Abnormal values were based on locally or published references. Ethics approval was obtained and all subjects gave written infomred consent.
Results: Data from 32 patients (median age 61, range 41-72, 15 females) were analysed. Median duration in years from diagnosis was 5.8 (range 1.9-11.3) and 29 (90.6%) had only autologous HSCT and 3 (9.4%) allogeneic HSCT. 7 (21.8%) had 2 HSCT procedures. Other treatments varied between patients and included thalidomide, bortezomib, vincristine, doxorubicin and lenalidomide. All patients had received multiple pulses of high dose steroids with chemotherapy and had been treated from diagnosis with bisphosphonates.
Clinical history and endocrine testing identified 5/32 (15.6%) patients were hypothyroid and 12/15 (80%) males hypogonadal. Synacthen tests were normal in all patients despite the heavy steroid pretreatment. In 4/15 (26.5%) females raised prolactin levels were noted. Body mass index was >25 kg/m^2 in 13/15 (86.6%) males and 5/15 (33.3%) in females. 2/27 (7.4%) had reduced B12. Ferritin levels were raised in 11/27 (40.7%). 17/29 (58.6%) had vitamin D insufficiency (<50 nmol/L) and 10 had deficiency (<30 nmol/L). 8/27 (29.6%) had raised age-matched BMD at the femoral neck with the remainder normal BMD.
Conclusion: In this sample of advanced stage but stable myeloma patients who had received HSCT and other intensive treatments, endocrine and nutritional abnormalities were common, but surprisingly bone density appears to be well preserved with modern anti-myeloma and bisphosphonate treatment strategies. Increased understanding and further study of 'late effects' in myeloma should help to optimise supportive care interventions with the aim of maintaining quality of life and prolonging survival in long term myeloma survivors.

P700

Platelet apoptosis measurement as a quality control of physicochemically treated apheresis platelet concentrates used for transfusion to patients after haematopoietic stem cell transplantation

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Background: Different methods of apheresis platelet concentrates (A-PLT) treatment, such as Photochemical treatment (PCT) with amotosalen and long-wavelength ultraviolet A (UVA) light and Γ irradiation (GI) with 25Gy, are used for transfusion safety providing. Platelets (PLTs) undergo apoptoticlike changes in response to chemical and physical stimulation, therefore quality control of treated A-PLT is necessary.
The aim of this study is to evaluate and compare the viability of A-PLT before and after physicochemical treatment (PLTs after PCT and GI) on the base of apoptosis marker. Methods: 32 samples of A-PLT were investigated before treatment, 16 - after PCT and 16 - after GI. PLT were prepared in 35% plasma and 65% platelet additive solution, pre-PCT yield was $2,5\text{-}6,0x10^{11}$ in volume 320±5 ml. A-PLT were treated with 150 μM amotosalen and 3,6 J/CM^2 UVA light or γ irradiated with 25Gy during the first 24 hours after collection. All samples were investigated by flow cytometry. PLT apoptosis was measured by phosphatidylserine (PS) exposure with FITC-labeled Annexin V. PS exposure at the PLT membrane surface of donors blood samples before apheresis was measured for comparative evaluation. All A-PLT were transfused to thrombocytopenic patients after haematopoietic stem cell transplantation.
Results: PS exposure at the PLT membrane surface of donors blood was not exceeded 5,6±4,2%. PS externalization at the membrane surface of A-PLT increased during apheresis

procedures and was detected on 12,3±5,3% PLTs. The degree of Annexin V binding was not changed significantly after PCT (12,6±4,9%). The percentage of apoptoticlike changed PLTs remained unchanged after GI and PS exposure was detected on 13,6±4,6% PLTs. Transfusions of physicochemically treated A-PLT demonstrated acceptable corrected count increments and clinical efficacy.
Conclusions: Apheresis, PCT and GI (25Gy) of A-PLT was not lead to clinicaly significant increasing of PLT apoptosis. Transfusions of these A-PLT may provide effective posttransfusion viability of PLTs.

P701
Supersaturated calcium phosphate rinse (Caphosol) in the management of mucositis after haematopoietic stem cell transplantation - single-centre experience
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Introduction: severe mucositis as a typical complication of myeloablative chemotherapy and/or total body irradiation (TBI) is still a problem in patients after hematopoietic stem cell transplantation (HSCT) causing pain, inadequate food intake and increasing risk of general infection. An attempt to decrease the intensity of mucositis by employment of supersaturated calcium phosphate rinse (Caphosol) in standard mucosals care was performed at our Department of Transplantation.
Goal: evaluation of the effect of Caphosol on life quality of HSCT patient measured as: degree of mucositis, opioid requirement, need for total parenteral nutrition (TPN) and length of hospital stay in comparison with historical control.
Material and method: Since Jan 2009 till Nov 2010 Caphosol was employed as standard care in 34 pediatric HSCT patients (8 autologous and 26 allogeneic). Historical control of 52 patients (31 autologous and 21 allogeneic) transplanted between 2005 -2008 was used to comparison. We have evaluated: number of days with TPN, days with usage of parenteral morphine, employment of antibiotic and antifungal drugs and length of hospital stay.
Results: (in table).
Conclusion: introduction of Caphosol has lead to decrease of frequency of high degree mucositis reflecting in decrease of usage of TPN and opioids.

Treatment group – No of patients	without Caphosol-52	with Caphosol-34	p
Degree of mucositis: No with stage III and IV (%)	31(59,6)	11(32,4)	0.013 s.s
Days On Morphine- mean	10,9	8,0	0.120 n.s.
Days on TPN-mean	14,9	9,9	0.010 s.s
Days with fever -mean	6,3	4,6	0.120 n.s.
Days of hospital stay – mean	33,7	29,7	0.370 n.s

P702
Effectiveness of a three-drug regimen of dexamethasone, palonosetron and aprepitant for the prevention of acute and delayed nausea and vomiting caused by high-dose therapy before haematopoietic stem cell transplantation
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Background and aims: A combination of a serotin antagonist, a corticosteroid, and a NK-1 antagonist is effective against nausea and vomiting due to highly emetogenic chemotherapy based on cisplatin. We test this three-drug combination in the prevention of nausea and vomiting due to high-dose therapy before hematopoietic stem cell transplantation (HSCT).
Patients and methods: The combination was based on aprepitant 125 mg p.o. day 1 chemotherapy and 80 mg p.o. day 1 and 2 post-chemotherapy , palonosetron 0.25 mg i.v. day 1 ± day 4 ± day 6 chemotherapy (according to the duration of chemotherapy), dexamethasone 8 mg i.v each day of chemotherapy and day 1 and 2 post-chemotherapy. Acute (during chemotherapy) and delayed (for 3 days after chemotherapy) nausea and vomiting were daily evaluated with a Study Diary which recorded frequency and intensity of nausea and vomiting, limitation of feeding and patient activity (TV watching, reading, phone using) and use of rescue medications.
Results: Twenty-one patients with a median age of 54 years (range 23-70) were prospectively evaluated. Conditioning regimens were busulphan-cyclophosphamide for acute leukemias (6 patients), melphalan 200 mg/mq for multiple myeloma (9 patients), BEAM (carmustine, cytosine arabinoside, etoposide, melphalan) for lymphomas (6 patients). Autologous (19 patients) or allogeneic (2 patients) peripheral SC were reinfused day 2 post chemotherapy.
Results: Overall 81 days in the acute period and 54 days in the delayed period were examined. Absence of vomiting was presented in 66/81 (81%) days in the acute period and in 36/54 (67%) days in the delayed period. Absence of nausea was achieved in 44/81 (54%) days in the acute period and in 23/54 (43%) days post-chemotherapy. Rescue therapy was administered in 12/81 (15%) days in the acute period and in 9/54 (17%) days in the delayed period. No limitation of feeding and daily activity was presented in 44/81 (54%) and in 46/81 (57%) days of acute period respectively and in 18/54 (33%) and in 22/54 (41%) days post-chemotherapy. Kinetics of engraftment and incidence of mucositis and infections did not differ in comparison with other 25 HSCT matched for baseline clinical characteristics.
Conclusions: We conclude that a three drug antiemetic regimen of aprepitant, palonosetron and dexamethasone was feasible and effective for the protection against both acute and delayed nausea and vomiting due to high-dose chemotherapy.

P703
Prevention of cytarabine-induced kerato-conjunctivitis by eye rinse in patients receiving high-dose cytarabine and total body irradiation as a conditioning for haematopoietic stem cell transplantation
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Introduction: We have previously reported a high incidence of kerato-conjunctivitis in patients receiving high-dose cytarabine following total body irradiation (TBI) as a conditioning for hematopoietic stem cell transplantation (HSCT) even under prophylaxis with topical corticosteroid. This study aimed to evaluate whether the addition of eye rinse during and shortly after cytrabine infusion, which was designed to efficiently remove cytarbine from the ocular surface, may further reduce the incidence of kerato-conjunctivitis in the same setting.
Patients and methods: Seventy-six patients who received cytarabine at a dose of 3 g/m² over 2 hours every 12 h for 4 days after receiving TBI (12 Gy) as a conditioning for hematopoietic stem cell transplantation were evaluated. All patients received βmethasone sodium phosphate eye drops. Twenty-three patients were further instructed to rinse their eyes with sterile saline every 10-15 minutes during and for two additional hours after the completion of each cytarabine infusion.
Results: Among the 23 patients with eye rinse, Grades 2-3 and 1-3 kerato-conjunctivitis were observed in 4 (17.4%) and 5 patients (21.7%), respectively. These incidences were significantly lower than those (35 (66.0%) and 41 (77.4%)) observed in 53 patients without eye rinse (P<0.001 and P<0.00001, respectively).

Conclusion: These results strongly suggest that eye rinse could effectively reduce the incidence and severity of cytarabine-induced kerato-conjunctivitis and improve the quality of life in HSCT recipients who receive high-dose cytarabine following TBI.

P704

Results of allogeneic haematopoietic stem cell transplant from Erciyes Capodoccia Transplantation Center: 11-year experience in centrall Anatolia

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Objective: In this retrospective study, it was aimed to perform analysis of transplantation outcomes of patients who underwent AHSCT, to evaluate factors contributing mortality on post-transplantation period and determine relative risk of death caused by these factors.
Patients and Method: Outcomes of 184 patients who underwent AHSCT between 1999 and January, 2010 in Erciyes Capodoccia Transplantation Center were retrospectively assessed. The patients underwent AHSCT were classified by considering the factors such as diagnosis, time of transplantation, health status at the time of transplant, time between diagnosis and transplantation, histocompatibility, donor-recipient gender compatibility, amount of CD4+ stem cell which was given, preparation regimes and risk status at the time of diagnosis. The groups were compared in terms of graft versus host disease (GVHD), recurrence, overall survival, early and perioperative mortality.
Findings: When compared to other groups, the overall survival rate was found higher in the patients with low risk acute leukemia (AL), the patients with acute lymphoblastic leukemia who underwent TBI-based transplantation, the patients with AL and lymphoma who underwent AHSCT at complet remission 1 phase. Relative mortality risk associated with transplantation due to any reason was given Table 1.
When confounding factors of transplant-related mortality were assessed by using multivariate Cox regression analysis, recurrent disease development was shown to be the most significant parameter affecting mortality. No significant relationship was found between mortality and age, gender, histocompatibility, preparation regime, donor gender compatibility and amount of CD4+ stem cells which was given.
Conclusion: Factors such as age, disease phase and prognostic features, time between diagnosis and transplantation, histocompatibility and donor-recipient gender compatibility are important parameters for risk assessment before AHSCT. Concurrence of these risk factors markedly increases probability of transplant-related complication and mortality. Furthermore, occurrence of acute and chronic GVHD and recurrent disease significantly increase the probability of transplant-related mortality.
In conclusion, it is very important for each stem cell transplantation center to determine risk groups for mortality and morbidity and outcomes in their own patient groups.

	Relative mortality risk associated with transplantation due to any reason [95 % CI]	p
AGE	0,997 (0,997 - 1,017)	0,756
SEX		
Male*	1	
Female	1,16 (0,75 - 1,79)	0,517
PHASE OF DISEASE		
Complet remission$_1$*	1	
Complet remission$_2$	2,67 (1,25 - 5,70)	0,011
> Complet remission$_2$	2,18 (1,12 - 4,20)	0,021
RISK STATUS (for acute leukemias)		
Low Risk*	1	
High Risk	2,47 (1,47 - 4,14)	<0,001
ACUTE GVHD		
No*	1	
Yes	1,78 (1,10 – 2,88)	0,019
CHRONIC GVHD		
No*	1	
Yes	1,92 (1,19 – 3,09)	0,008
RELAPS		
No*	1	
Yes	3,12 (2,02 – 4,82)	<0,001
TIME BETWEEN DIAGNOSIS AND TRANSPLANTATION		
< 12 Months*	1	
>12 Months	1,48 (0,95 – 2,30)	0,08
CONDITIONING REGIMES		
Myeloablative*	1	
Reduced intensity	1,23 (0,78 – 1,94)	0,381
HLA TISSUE COMPATIBILITY		
Full Match Related*	1	0,709
Mismatch / Unrelated	1,12 (0,62 – 2,03)	
DONOR-RECIPIENT GENDER COMPATIBILITY		
Compatible *	1	
Female donor-Male recipinet	1,07 (0,67 – 1,70)	0,788
AMOUNT OF GIVEN STEM CELLS	0,97 (0,85 – 1,11)	0,667
ALL CONDITIONING REGIMES		
TBI Based Regimes	1	
Other Regimes	3,63 (1,23 – 10,66)	0,019

Cox-regression analysis, "*" means that Reference Group

P705

Efficiacy and safety of deferasirox in patients undergoing stem cell transplantation in post-transplant period

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Introduction: Iron overload is one of the important causes of mortality and morbidity in patients whom underwent both allogeneic (Allo-SCT) and autologous stem cell transplantation. Excessive iron accumulation results in tissue damage and organ failure presumably via generation of free radicals, resulting in oxidative damage and organ dysfunction (e.g. hepatotoxicity, cardiotoxicity, endocrine dysfunction).
Patients and methods: A total of 23 patients with hematological disorders whom underwent ASCT between February 2006-August 2009 were retrospectively investigated. Inclusion criteria of the study consisted of: 1) Hyperferritinemia (ferritin> 1000 ng/ml), 2) Liver enzyme abnormalities attributed to iron overload or patients who had biopsy proven hemochromatosis. Liver biopsy was performed to patients whose LFT showed no amelioration for 7 days and patients whose LFT continued to deteriorate.
Results: A total of 23 consecutive patients with hematological disorders given human leukocyte antigen (HLA)-matched allogeneic HSCT were retrospectively evaluated. All of them have had hyperferritinemia and transfusion-associated iron overload.14 of 23 patients were female (61%) while 9 were male (39%).15 of the patients (65%) were diagnosed as acute myelogenous leukemia (AML) and 8 of them (35%) were acute lymphoblastic leukemia (ALL).The median age was found 31 years 17-54) and median hemoglobin value was 11,9 gr/dl (7-16). The median time to start deferasiroks in the post-transplant period was 115 days.The median number of transfusion was 23 (11-67. Median treatment time with deferasiroks was 90 days.Only 11 patients (47%) had undergone transcutaneous liver biopsy due to the fact that it is an invasive procedure and the patients' unwillingness.The biopsy records revealed that 6 (26%) of the patients had grade 4 hemosiderosis, 3 of them grade 3 (13%) while 3 patients had grade 2 (13%) hemosiderosis. The grade of hepatic iron overload was correlated with the levels of pre-treatment serum ferritin levels. The liver enzymes, especially ALT and bilirubins were significantly reduced after the treatment ($p<0,05$). The deferasiroks treatment reduced pre-transplant ferritin levels in a period of 90 days below the level of 1000 ng /mL. This result was found statistically significant $p<0,005$.
In conclusion, deferasiroks seems to be a safe and effective oral treatment method for reducing iron overload at a dose of 20 mg/kg in post-transplant period combined with or without phlebotomy.

P706

Duration of reinfusion is not associated to engraftment in autologous stem cell transplantation

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Autologous stem cell transplantation (ASCT) is a common procedure for haematological malignancies, and it is now

performed mostly with DMSO-cryopreserved peripheral blood stem cells (PBSC), and different modalities of thawing and reinfusion of PBSC are used. A correlation between engraftment and the time elapsed from PBSC thawing and the end of reinfusion could be hypothesized. We reviewed the ASCT performed at our institution to evaluate the correlation between engraftment and timing of PBSC thawing and reinfusion.
Data from 41 ASCT procedures performed in 38 patients (pts) since February 2007 were retrospectively analysed. Primary disease was myeloma in 16 pts, non-Hodgkin's lymphoma in 13 pts, Hodgkin's lymphoma in 5 pts, and acute myelod leukemia, amyloidosis, chronic lymphocytic leukemia, and Evan's syndrome in 1 pt each. Conditioning regimens used were high-dose melphalan (140 or 200 mg/sqm) in 20 cases, BEAM in 19 cases, and BUCY2, and cyclophosphamide-ATG in 1 pt each. Median age at ASCT was 59 years (range 33-69), and 18 pts were male. All pts received pegfilgrastim 6 mg on day +1. Thirty-six procedures were first ASCT, four were second ASCT, one was a third ASCT. Median number of infused CD34+ cells was $5.13x10^6$/Kg (range 1.83-11.54); units of PBSC were one in 18 cases, two in 21 cases, three in 2 cases. Viability at cryopreservation was tested in 33 PBSC units, with a median value of 91% (range 70-99). Five pts experienced CMV reactivation after ASCT. Median time to engraftment was 10 days (range 9-18) for neutrophils (PMN) and 14.5 days (range 10-33) for platelets (PLT). One patient, now at day +50, did not engraft for PLT, and is still transfusion-dependent. Engraftment at +100 was evaluable in 33 cases; at this time median PMN count was 2200/mmc (range 500-4200), and median PLT count was 134000/mmc (range 26000-244000). Age at ASCT, number of previous lines of chemotherapy, number of CD34+ cells infused, viability, and CMV reactivation showed no correlation with time to engraftment and cell counts at +100. Considering a time of ten minutes between the end of thawing and the end of reinfusion as a threshold, both engraftment and cell counts at +100 did not correlate with duration of reinfusion.
In conclusion, our data do not suggest that timing of reinfusion might affect engraftment. Considering the small number of pts included and the retrospective nature of our study, a larger multicentric study may probably solve this question.

P707
Allogeneic haematopoietic stem cell transplantation following liver transplantation
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Haematopoietic stem cell transplantation (HSCT) following solid organ transplantation was rarely reported. To our knowledge there exist only 5 reports on HSCT in aplastic anemia between 3 months and 4 years after orthotopic liver transplantation (LT) for non-A, non-B, non-C hepatitis. Here we report for the first time on allogeneic HSCT 15 years after successful LT in a 33-year old patient with unclassified myeloproliferative disorder.
At the age of 18 the patient received LT due to solvent-related acute-toxic liver failure. Six respectively eight years later he developed anaemia and thrombopenia. 15 years after LT he complained about loss of performance, weight loss of 15kg within 7 months, night sweat and episodes of bleeding with epistaxis and haematoma. While the liver function was unremarkable, the bone marrow biopsy displayed features of both myeloproliferative disorder and myelodysplastic syndrome. In the absence of defined molecular markers (e.g. JAK2 and BCR/ABL) an unclassified myeloproliferative disorder was diagnosed and a therapy with hydroxyurea started. After conditioning with FLAMSA (fludarabine, amsacrine, total body irradiation, ATG and cyclophosphamide) the patient received HSCT from mmVUD. GVHD-prophylaxis consisted of ciclosporine. On day 2 after HSCT he developed cholestasis with an increase of alkaline phosphatase (max. 160U/l), bilirubin (max. 194 µmol/l) and the γ-glutamyl transferase activity (max. 197U/l) while the liver transaminases remained in normal range. Persistent fever, which was unresponsive to broad spectrum antibiotics as well as caspofungin occurred first at the day of transplantation. High-resolution CT scan showed signs of pulmonary mycosis and antifungal therapy was escalated. Microbacteriological blood culture were negative. The engraftment was delayed: leukocytes >1Gpt/l day +42 neutrophils > 0.5 Gpt/l day+ 41, haemoglobin > 6,0 mmol/l day +46, thrombocytes >50Gpt/l day +46. Currently the afebrile patient was discharged from hospital 42 days after HSCT with no signs of GVHD and he reports on an improved performance status compared to onset of clinical symptoms.
There are several significant concerns when considering an HSCT following SOT and especially the likelihood of severe hepatic toxicity was anticipated in this patient. However, the case demonstrates that allogeneic HSCT is technically feasible and the course will be updated at the EBMT-meeting.

P708
CLL patients post NST: increased demands on inpatient and ambulatory care
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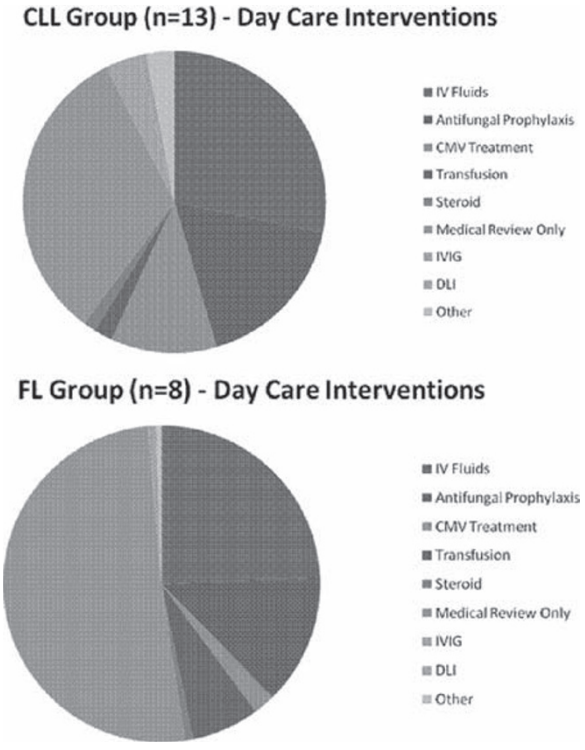
Hypothesis: Patients undergoing Non-Myeloblative Stem Cell Transplant (NST) for Chronic Lymphocytic Leukaemia (CLL) require far greater input from day care settings and need for readmission to hospital in the post transplant period when compared to a similar population.
Methods: A retrospective chart review of all patients who received an NST for CLL (n=13) from 2003 was performed. We assessed the level of activity at 0-3mth; 3-6mth; and 6-12months intervals post NST. The results were then compared to a similar population, namely patients with Follicular Lymphoma (FL) who underwent an NST (n=8).
Results: The mean transplant length of stay for the CLL group was 37.15 days, in comparison to 33.75days for the FL group. However, the CLL group had a significantly more recidivist rate, 35 v 17 (Mean 2.69 v 2.125). Readmission rates were similar at 0-3mth and 3-6mth interval between the 2 groups. However,



the CLL group had a considerable increase in hospitalisation at the 6-12mth interval (Mean 1.55 V 0.5).
In relation to Day Care visits, Patients with CLL required a greater number of outpatient attendances (632 V's 344). As with readmission rates at the 6-12 mth interval, the CLL group required significantly more visits at the 6-12mth period (Mean 19.36 v 12.66). When comparing the 2 groups, we observed that the CLL group required much greater intervention in the Day Care setting (Chart below), Mean 57.15 v 45.12 per patient. This was even more remarkable when visits for Medical Review only was excluded, Mean 38.62 v 22.12, respectively. Mortality rates were also found to be higher in the CLL group (5 v 1).
Conclusion: We have clearly demonstrated that patients undergoing NST for CLL require significantly greater post transplant care than a similar population. Of interest, the period of 6-12mth was the interval with the greatest requirement for both inpatient and outpatient care. These results have implications on future planning and identification of post transplant needs in this patient population.

P709

Assessment of the EBMT risk score in Japanese patients who received allogeneic stem cell transplantation in a single centre

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Background and objectives: Several pre-transplant scoring systems are available to predict outcome in allogeneic hematopoietic stem cell transplantation (allo-SCT). The EBMT risk score was initially established for chronic myelogeneous leukemia patients based on registry data and was recently validated in second registry analysis for other hematologic malignancies. We evaluated whether the EBMT score predicted overall survival (OS) at a Japanese transplant center.
Design and methods: We performed a retrospective study in a cohort of 83 patients with acute myeloid leukemia (AML; n=33), acute lymphoblastic leukemia (ALL; n=12), myelodysplastic syndrome (MDS; n=16), chronic myelogenous leukemia/myeloproliferative disease (CML/MPD; n=5), non-Hodgkin's lymphomas (NHL; n=13), and adult T-cell lymphoma/leukemia (ATLL; n=4), who were transplanted from a human leucocyte antigen (HLA)-matched sibling (n=7), an unrelated bone marrow donor (n=62) and unrelated cord blood (n=14) after myeloablative conditioning regimens (n=39) or reduced intensity regimens (n=44) between 2005 and 2009 at our center. Median age was 45 (range: 16-65).
Results: According to EBMT score, 5 patients had a score 0-3, 62 patients had a score 4-7 and 16 patients had a score >8. Two-year OS was 100%, 42% and 15% in the low (sore 0-3), intermediate (score 4-7) and high-risk (score >8), respectively (p=0.025).
Conclusion: Our single-center study suggests that the EBMT is a good predictor of 2-year survival after allo-SCT in all hematological disease categories and is independent of stem cell source or conditioning regimen.

P710

Donor cell derived acute myeloid leukaemia in a patient previously submitted to allogeneic stem cell transplant for acute myeloid leukaemia

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A 27 years old man was submitted to Matched Unrelat Donor (MUD) Hematopoietic Stem Cell Transplant (HSCT) for Acute Myeloid Leukaemia (AML) in first complete remission. AML characteristic at the onset were: normal caryotype, FLT3-ITD and NPM1 gene mutations respectively positive and negative, phenotype: CD33+/CD13+/CD34+, FAB: M1, hyperleukocytosis and a retinic localisation. Transplant procedure did not presented significant complications except an hemorrhagic cystitis. Also the post-transplant follow-up was regular without Graft versus Host Disease nor other complications. Immunosuppression was regularly tapered and 18 months after HSCT it was stopped; subsequently the patient enjoyed a normal life with periodic clinical controls. Seven years after HSCT a decrease in erythrocytes an leucocytes count was observed: a bone marrow aspirate and a biopsy showed a blast infiltration arising about 30% compatible with AML. FAB, immunophenotypic and cytogenetic features were the same of the onset, but differently NPM1 resulted mutated (NPM1-A mutation). Instead FLT3-ITD became negative.
A chimerism status analysis was performed on the same marrow sample employing Short Tandem Repeats method. In our laboratory a panel of 15 STRs loci plus Amelogenin locus is usually employed: it showed a full donor chimeric status. The analysis was repeated on a second marrow sample and it confirmed the previous data.
We concluded that after seven years since HSCT, the patient developed a second AML, NPM1-A mutated, derived from donor cells. We searched NPM1-A mutation on the sample of donor marrow DNA stored at the time of the transplant and it resulted negative. We also contacted the clinical referees of the marrow donor centre and they confirmed that the donor enjoys a good health. This two evidences support the hypothesis that the donor derived AML developed in the host after HSCT.
We here describe this case for its rarity. In fact the onset of a second neoplasm in individuals submitted to HSCT is not a rare event and the principal causes are the toxicity of chemo and radio-therapy and the lack of immunologic surveillance. But in this case the second neoplasm grew in the host but originated from donor cells. This and eventually other similar cases may be studied in order to obtain a major comprehension of the mechanism of immunologic surveillance and cell donor uptake in the recipient of HSCT.

P711

Complete neurologic and cognitive recovery after plasmapheresis in a patient with chronic inflammatory polyneuropathy after allogeneic bone marrow transplantation

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Case report: The patient (male, caucasian, 57 years) was diagnosed in 2009 with an acute myeloid leukemia (AML) (FAB: M1). He received standard induction chemotherapy with cytarabine, daunorubicin and etoposide and a second induction chemoherapy with MIDAC (mitoxantrone, cytarabine) and a following consolidation. Six months later, an allogenic bone marrow transplantation (BMT) after conditioning chemotherapy with FLAMSA-protocol (fludarabin, cytarabine, amsacrine) was performed.
Shortly after the BMT the patient developed progressive polyneuropathy of the lower legs and hypoaethesia on both feet. Five months later the patient developed a severe dementia (mini-mental-state-examination: 4) with changes in personality and urinary retention. All magnetic radiographic images (MRI) and computed tomographies (CT) of the brain and spine showed no specific pathologies. The spinal fluid analysis showed slightly elevated cells with high protein levels and lymphocytic cells. All viral (cytomegalovirus, herpes simplex virus 1 and 2, varicella virus, ebstein-barr-virus) and bacterial diagnostics in the liquor were negative. The somatosensory evoked potentials (SSEP) were pathologic in concern of the lower right extremities in form of conduction disturbance of the somatosensoric tract.
Due to severe worsening of the neuropsychiatric status and the results that were highly suspicious for chronic inflammatory polyneuropathy, the patient received ten cycles of plasmapheresis.

During plasmapheresis the patient showed a significant improvement of the neuropsychiatric symptoms. The cognitive status improved to almost normal (mini-mental-state-examination: 28).
Conclusion: Autoimmune phenomenon after allogenic BMT, like chronic inflammatory polyneuropathy, have great variability in symptoms and presentation and are challenging to diagnose and treat. Plasmapheresis is a save and highly efficient treatment for patients with unclear persisting autoimmune neuropathy after BMT.

P712
PET-evaluation of extramedullary relapse following allogeneic stem cell transplantation in haematologic malignancies
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Introduction: Extramedullary relapse following allogeneic stem cell transplantation (SCT) in hematologic malignancies is a rare event for which no standard approach exists.
Generally, extramedullary manifestation in myeloid or lymphoid malignancies can lead to a variety of symptoms so that the diagnosis of relapse might be difficult to establish.
We report on 3 patients with extramedullary relapse of acute myeloid leucemia (AML), acute lymphoblastic leucemia (ALL) and chronic myeloid leucemia (CML), respectively, after allogeneic SCT diagnosed by PET-CT imaging. All patients presented with unspecific symptoms so that initially other causes were suspected. One female patient with refractory AML presented with dysphagia and gastric pain after successful allogeneic SCT. An urgent esophagoduodenoscopy was performed, which revealed a submucosal tumor located at the gastric antrum. Gastric surgery revealed myeloid sarcoma and a then performed PET CT imaging showed multiple lesions in bones, liver, spleen and in the whole gastrointestinal tract. In contrast, no hematologic relapse was diagnosed.
A female patient with ALL with also a history of allogeneic SCT presented with continuous pain in her left knee, where no pathologic findings were seen in the conventional x-ray- examination. PET-CT imaging showed multiple bone lesions with high levels of pathologic glucose metabolism. A biopsy revealed extramedullary relapse and PET CT imaging was performed to evaluate the further clinical course following chemotherapy.
A female patient with CML blast crisis after successful allogeneic SCT presented with ataxia and polyneuropathia and initially, meningeosis leucaemica was suspected. PET CT imaging revealed multiple lesions in the spinal cord as well as multiple bone and skin lesions. Myeloid sarcoma was confirmed by skin-biopsy.
Thus, extramedullary relapse after allogeneic SCT is a rare event that should be considered if unspecific symptoms occur, even though there is no hematologic relapse. PET CT imaging should be performed in order to establish the diagnosis.
Conclusion: In summary, extramedullary relapse in hematologic malignancies after allogeneic SCT should be suspected if unspecific symptoms occur. PET CT imaging is a reliable method to confirm the diagnosis. In addition, PET-CT imaging can also be used to evaluate the further course following any specific treatment.

P713
Immunoadsorption in a hyperimmunized patient undergoing mismatched allogeneic haematopoetic stem cell transplantation
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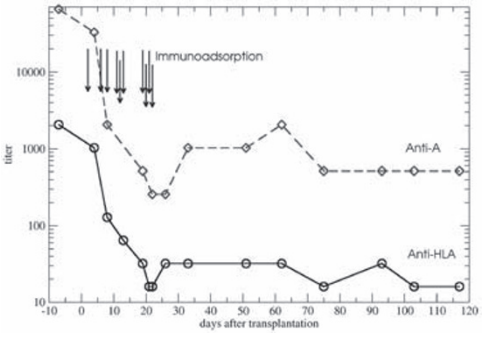
Objectives: A 37-year-old female patient blood group O, underwent hematopoetic stem cell transplantation for tyrosine-kinase inhibitor refractory chronic myeloid leukaemia after a second blast crisis. As no human-leucocyte-antigen (HLA)-ident donor was available, a DQB1-mismatched, major ABO incompatible donor, blood group A, was accepted. The patient was hyperimmunized with 96% panel reactivity against HLA class I antigens, and against multiple HLA class II antigens including the donor's mismatch. The anti-A titer was extremely high. A regimen of immunoadsorption and suppression of antibody synthesis was used to bridge over the refractory thrombocytopenic period after grafting.
Methods: The patient received Amasacrine + Fludarabine (FLAMSA) d -12--9, Busilvex (8 mg/kg d -6- -4), Cyclophosphamide (60 mg/kg d -3- -2), ATG Fresenius (20 mg/kg d -4- -2), and for prophylaxis of graft-versus-host disease CSA and MMF. 10 sessions of immunoadsorption (IA) were performed using TheraSorb®-Ig flex columns from day +4 to day +22. Anti-A1 titers were determined using the DiaMed Micro typing gel method. The titer endpoint was the reciprocal of the highest dilution demonstrating agglutination. Titers of HLA class I antibodies were similarly monitored by the monoclonal antibody immobilization of platelet antigens assay (MAIPA). HLA class I and class II antibodies were specified using the Luminex® technique.
Clinical course: Prior to transplantation, the patient had received high dose immunoglobulin and corticosteroids for thrombocytopenia, but without any effect. Starting day -4, HLA-matched platelets were given with no increment at all, despite 500 mg Rituximab on day -1. After IA therapy was started, the HLA antibody titer decreased from 1024 to a minimum of 16. This was paralleled by anti-A1-titer reduction from 65536 to 256. The first successful platelet increment occurred on day +11, neutrophil engraftment on day +16, and platelet engraftment on day +23. IA was tolerated well. After discharge, titers rebounded temporarily up to 32 for anti-HLA and 2048 for anti-A1. At day +120, the patient is clinically stable with residual peripheral Ph+ cells.
Conclusions: A in combination with immunosuppression contributed to engraftment in a HLA class II mismatched situation by removing HLA antibodies. Indirect monitoring of IA efficiency was possible by isoagglutinin titration. IA may be considered during allogeneic stem cell transplantation when no suitable platelet product is available.



P714
Quality of life of patients with haematological malignancies or disorders before and after haematopoietic stem cell transplant
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Treatment for haematological malignancies is usually effective but has unavoidable side effects. The conditioning chemotherapy regimen of transplant is one of the most intensive chemotherapies. Do survivors of transplant have better quality of life (QOL)?
Objective: We aim to assess the level of functioning, quality of life, level of symptomatology of haematological patients before and after undergoing stem cell transplant.

Methods: Consecutive patients undergoing stem cell transplant from November 2009 to May 2010 were assessed using QLQ-C30 (self assessment questionnaire) on admission to the transplant ward just before undergoing stem cell transplant. Written consent was obtained. Demographic data as well as disease details and status of patients are collected at assessment. Another assessment of the QLQ-C30 was done after the transplant (from D+30 until D+180). The QLQ-C30 was scored according to the global health, functional level and symptomatology.
Results: A total of 29 patients answered the survey.
Global health status and functional abilities (physical, role, cognitive, emotional, social) of our patients seemed to be lower than the general population. Our patients seemed to have similar global health, physical and emotional status but lower role function and also cognitive function compared to the "all cancer" reference population. Global Health Status was similar in pre and post allogenic transplant patients with only autologous transplant patients appeared to have better scores post transplant. In the early post transplant period, most of the allogenic transplant patients had worse scores while most of the auto pts have better scores.
Conclusion: Haematology patients who are planned to undergo transplant have similar QOL as other cancer patients. Allogenic stem cell transplant patients have a worse QOL in the early post transplant period. Autologous transplant patients seemed to have better QOL in the early post transplant period.

Total surveyed (Pre transplant) = 29
Auto 13, Allo 15, Cord 1
Gender = 20 female, 9 male
Marital status = 13 married, 16 single
Children = 7 have children, 22 no children
Race = 12 Chinese, 16 Malay, 1 Indian
ECOG performance status
0 = 4 patients
1 = 23 patients
2 = 1 patient
3 = 1 patient
Median Age = 28.3 years
Education Level = 16 Secondary school, 13 tertiery education
Diagnosis
Aplastic Anaemia = 2
Acute Lymphoblastic Leukaemia = 6
Acute Myeloid Leukaemia = 8
Acute Promyelocytic Leukaemia = 1
Chronic Myeloid Leukaemia = 2
Diffuse Large B Cell Lymphoma = 1
Follicular Lymphoma = 1
Hodgkin Lymphoma = 5
Myelodysplastic Syndrome = 1
Multiple Myeloma = 2
Post transplant assessment (Total surveyed = 13)
At median 126 days post transplant
Range 41 – 158 days

(Median scores)	Global health Status	Functional Scale – Physical Function	Functional Scale – Role Function	Functional Scale – Emotional Function	Functional Scale – Cognitive Function	Functional Scale – Social Function
Normal population	75	100	100	83.3	100	100
All cancer	66.7	80	80	75	83.3	83.3
All pre transplant(29)	66.7	80	66.7	66.7	66.7	66.7
All post transplant(13)	66.7	80	83.3	83.3	66.7	66.7

P715
Stem cell transplantation: a 10-year single.centre experience
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Stem cell transplantation is the treatment of choice for patients with hematological malignant diseases. Aim of this study is to evaluate the results in 10 years experience with this procedure in our center. During a this period we have treated 195 patients with different malignant hematological diseases.107 male, 88 female with median age of 34 years. Allogeneic sibling transplantation were performed in 56 patients, and autologous transplantation in 139 patients. According to diagnose: AML:91 ALL:10 CML:7 CLL:1 MP:1 NHL:20 HD:27 MM:34 AA:2 Ewing sarcoma: 1. Bone marrow was used as a source of stem cells in 28, PBSC in 168 patients. Conditioning regimen consisted chemotherapy with: Bu-Cy2,Bu-Cy-Mel,BEAM, high dose Melphalan, high dose ICE,Flu/Mel. Engraftment was reached on day +12 (10-24). Median value of MNC in BMT was 3,8x10/Kg (2,5-4,5), while in PBSCT MNC was 4,1x10 (2,8-12,0). TRM in allogeneic recipients was 16%, with non-relapse mortality 10%, and in autologous recipients TRM was 3,8% with non-relapse mortality 2,0%. Primary disease was cause of death after transplantation in 40% in allogeneic, and 66% in autologous transplantation. 35% of allogeneic and 28% of autologous transplantation were transplanted in active disease.

P716
First Russian experience of calcium phosphate mouth rinse usage for treatment of children with oral mucositis undergoing haematopoietic stem cell transplantation
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Objective: High-dose chemotherapy administered as conditioning regimen prior to hematopoietic stem cell transplantation (HSCT) leads to injury or disruption of oral and gastro-intestinal mucosa. Mucositis is associated with increased risk of infections, mortality of patients (pts), duration and doses of morphine, duration of hospitalization, higher costs of treatment. As previously described, Caphosol, a neutral, super-saturated, calcium and phosphate solution for mouth rinse, is effective and safe for prevention of oral mucositis (OM) in adults undergoing HSCT and in head and neck cancer pts receiving chemo- and radiotherapy. However, the effect of Caphosol on OM in children is not yet established. We decided to first evaluate efficacy, safety and tolerability of Caphosol added to standard OM management in children with OM after HSCT.
Methods: Five children (4-16 years; median 13) with AML (n=2; allo-HSCT), neuroblastoma (n=2; auto-HSCT), Ewing sarcoma (n=1; auto-HSCT) were included. All pts received high-dose chemotherapy before HSCT and had OM grade 1 (n=4) or 4 (n=1) before Caphosol administration. Pts used Caphosol rinse four times daily, 30 ml each time. The OM was assessed according to Oral Mucositis Assessement Scale (OMAP) published by Sonis et al. in 1999.
Results: We detected the regression of OM in all pts (8-15 days; median 12). The duration of morphine administration was 0 (refusal by the patient), 8,10,12, and 15 days, with progressive decrease of the dose. Four of 5 pts had pain decrease during first hours after first Caphosol administration. The fever with no positive microbiology tests has been developed in 3/5 pts with duration of 3, 13, and 15 days. No adverse events nor bad taste or other unpleasant sensations on behalf of pts have been observed.

Conclusion: Our preliminary findings suggested that calcium phosphate regimen (Caphosol) administered in addition to standard OM regimen ameliorates the OM in children with severe OM, and that this solution for mouth rinse should be evaluated in preventive setting. Now we are planning to conduct a randomized trial to evaluate Caphosol in prevention of OM in children undergoing HSCT.

P717

Immuno-adsorption treatment for ABO mismatch allogenic stem cell transplants: a single-centre experience

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Objectives: Between September 2009 and September 2010 fourty three stem cell transplants were performed at Guy's and St Thomas' Hospital. Of these twenty five patients underwent allograft transplants. Of those three patients were offered the glycosorb immunoadsorption treatment for ABO mismatched stem cell transplants. We investigated the differences between the pre and post anti a or anti b titres in the patients undergoing ABO mismatched stem cell transplants.
The objective is to look at the effect the immunoadsorption had on the anti a or b titres and the continued effect this had for the patients and their haemolysis post transplant.
Methods: The patient group was made up of three males and no females with a median age of fifty seven years. The immunoadsorption treatment was offered to patients with an anti a or anti b titre higher than 1:128.
Two patients with an anti a titre and one with an anti b titre.
The column used was a 'Glycosorb' column and the patients recieved the immunoadsorption treatment on Day -1 of their conditioning treatment before stem cell return on Day 0.
Results: Patient one recieved one immunoadsorption treatment with a baseline anti a titre of 1:128 and a post anti a titre of 1:64.
Patient Two recieved two immunoadsorption treatments over two subsequent days. His baseline anti b titre was 1:512 lowering to 1:256 post day 1 immunoadsorption and 1:64 post day 2 immunoadsoption.
Patient three recieved two immunoadsorptions. His anti a titre baseline was 1:512. After first treatment acheived 1:256. On the second day after treatment acheieving 1:64.
Conclusion: Each patient when recieving immunoadsorption treatment decreased their anti a or b titres within a range to complete their stem cell transplants.
Two of the three patients needed two immunoadsorption treatments on subsequent days. At present only one patient has developed haemloysis complications following their allograft transplants.

Minimal residual disease, Tolerance, Chimerism and Immune reconstitution

P718

Quantitative monitoring of mutated NPM1 enables early detection of impending relapse in patients with acute myeloid leukaemia following conventional chemotherapy and allogeneic stem cell transplantion

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Objective: Relapse of disease remains the major cause of treatment failure in patients with AML, even after allogeneic stem transplantation (SCT). Early detection of minimal residual disease (MRD) using molecular techniques could enable early preemptive therapy, but so far, the number of suitable markers is limited. More recently, mutations of the nucleophosmin gene (NPM1) have been described. These mutations are among the most common changes in adult AML and have shown their potential for MRD detection. However, only few studies on NPM1-based MRD detection have been published. In this study we investigated the suitability of NPM1 as MRD-marker in a large cohort of AML-patients (pts).
Patients and methods: 184 NPM1-mutant AML pts (median age 53.5 years (range, 21-81), treated in protocols of the study alliance leukemia (SAL) were prospectively monitored. We developed an optimized assay for the sensitive detection of the three most common NPM1-mutations using a Real-Time-Q-PCR with locked-nucleic acid (LNA) based primer-probe design. The threshold for molecular relapse (mol-Rel) was defined as a 10-fold increase in NPM1 transcript compared to the lowest level achieved or levels > 1%. Molecular non- or partial-responder were defined as pts without significantly decreasing NPM1 transcript levels or levels > 1% after completion of induction and consolidation.
Results: We studied 184 patients having one of the three most common NPM1-mutations, A (N=156), B (N=17) and D (N=11). A total of 1661 samples (978 BM; 683 PBL) were analyzed, the median number of samples per patient was 7 (range, 3-55), the median molecular follow-up was 453 days (99-1703 days). 65 of the patients (35.3%) had undergone SCT (12 auto SCT, 53 allo SCT), an FLT3-ITD mutation was present in 68 pts. (37%). According to our criteria, 18 pts were defined as molecular non-responders and non of them achieved a durable CR. In 28 pts with hematological relapse (hem-rel) and sufficient molecular follow-up, the rise of MRD preceded the hem-rel by a median of 66 days (range, 0-313 days). Out of 121 pts without mol-rel only one patient relapsed ($p < 0.001$). The patterns of MRD in patients post allo-SCT showed obvious differences compared to patients with chemotherapy.
Conclusions: In conclusion, our data indicate that NPM1 mutations are suitable markers for MRD analyses allowing early detection of recurrent disease. The use of this marker might enable preemptive therapy using DLI or targeted treatment.

P719

Up-regulation of TLR2 expression on G-CSF-mobilized peripheral blood stem cells is responsible for their rapid engraftment after allogeneic haematopoietic stem cell transplantation

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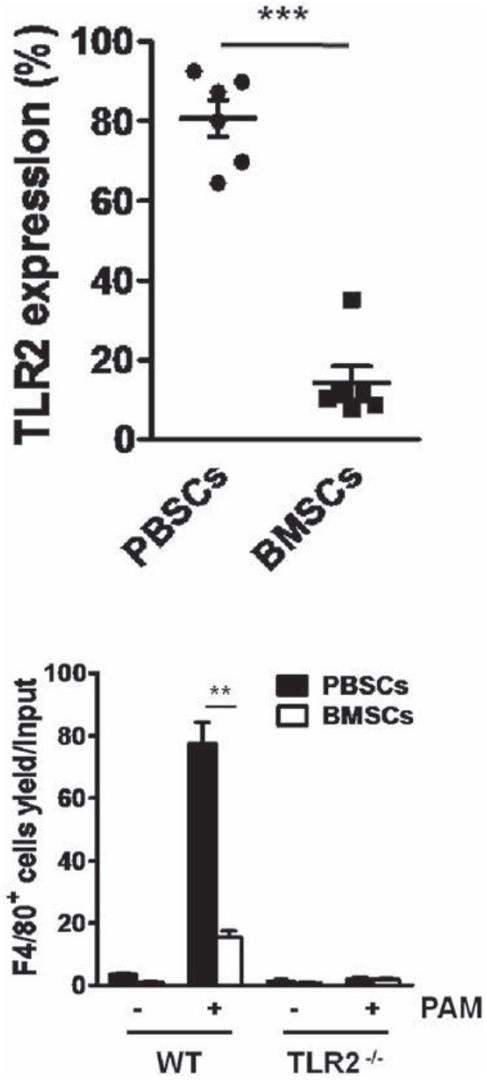
Granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSCs) are more frequently used as the cellular source in allogeneic hematopoietic stem cell transplantation (HSCT) than bone marrow stem cells (BMSCs) because they promote more rapid engraftment and immune reconstitution. However, the underlying mechanism for this is not fully understood. Here, we investigated the role of Toll-like receptor 2 (TLR2) on PBSCs in promoting rapid engraftment after allogeneic HSCT. We found that PBSCs highly expressed TLR2 in comparison to BMSCs, and TLR2 was directly induced by G-CSF signaling. Treatment with the TLR2 ligand, Pam3CSK4 (PAM), more efficiently induced myeloid differentiation of PBSCs than BMSCs. Similarly, endogenous TLR2 ligands from the serum of recipients of allogeneic transplantation more rapidly stimulated myeloid differentiation of PBSCs compared with BMSCs. PAM treatment of TLR2-deficient syngeneic recipient mice transplanted with PBSCs resulted in significantly elevated numbers of PBSC-derived myeloid cells and spleen colony formation compared with controls. Our results demonstrate that TLR2 signaling in PBSCs correlates with their ability to rapidly differentiate into myeloid cells, resulting in improved engraftment. Thus, TLR2 may be a novel target for increasing the efficiency of allogeneic HSCT by overcoming engraftment failure or delayed engraftment.

[P719]



P720

Low levels of T-cell receptor excision circles early after allogeneic stem cell transplantation is predictive of inferior outcome

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Background: T-cell function is defective for a prolonged period after allogeneic stem cell transplantation (ASCT). This can have a significant clinical impact by increasing susceptibility to infections and possibly also by delaying development of tolerance. One way to assess thymic output of T-cells is by measuring the level of T-cell receptor excision circles (TREC).
Methods: This study included 210 patients between the ages 0.5-69 years. All had been treated due to haematological malignancies and all were alive >3 months post ASCT. TREC levels were measured retrospectively at time-points 2, 3, 6, 9, 12, and 24 months post-ASCT, using a quantitative real-time PCR protocol. The amount of TREC in CD3+ cells was expressed as the ratio between TREC and the house-keeping gene GAPDH.
Results: TREC levels increased with time and the largest increase was seen between 3 and 6 months post ASCT. Younger age was significantly correlated with higher TREC levels after ASCT. The use of anti-thymocyte globulin correlated with lower TREC levels early (≤6 months) after ASCT. The use of bone marrow as graft, however, was significantly correlated with higher TREC levels as compared to peripheral blood stem cell grafts late (>6 months) after SCT.
TREC levels neither correlated with factors like diagnosis, intensity of the conditioning regimen, nor with clinical outcome like acute and chronic GVHD, and relapse incidence. However, when children were analyzed separately, acute GVHD II-IV and the use of irradiation in the conditioning regimen were both correlated with lower TREC levels.
The population was divided into two groups based on TREC levels at 3 months post ASCT. The group with levels above median showed a superior long term overall survival of 80% as compared to 56% (p=0.002). Accordingly, there was a significant difference in transplantation related mortality (TRM) between the two groups; 7% vs. 21% (p=0.01). This increased mortality could mainly be accredited to an increased incidence of fatal infections; 2% vs. 11% (p=0.01).
Conclusion: Our results offer further evidence that thymic output indeed has an important role in reconstituting the normal T-cell repertoire after ASCT. Patients with a preserved ability to produce newly differentiated T-cells seem to have an advantageous position in regard to warding off infections. We could further show that selection of conditioning regimen and graft source can have an impact on TREC levels, and subsequently affect outcome.

P721

WT1 transcripts kinetics predicts AML relapse after allogeneic haematopoietic stem cell transplantation: need for a monthly monitoring in high-risk patients

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Introduction: several approaches have been used in AML and MDS patients (pts) in complete remission (CR) to detect minimal residual disease (MRD) and predict the risk of relapse. The Wilms' tumor gene 1 (WT1) is over-expressed in > 80% of AML and advanced MDS, making this molecule an ideal marker for MRD monitoring. We analysed the kinetics of WT1 quantitative expression after allogeneic hematopoietic stem cell transplantation (HSCT) in pts at high risk of relapse.
Aim: to correlate the kinetics of WT1 expression in bone marrow (BM) of AML/MDS pts with relapse occurrence after HSCT.
Materials and methods: between 12/2007 and 10/2010, 57 pts (46 AML and 11 MDS) received HSCT (13 MRD, 18 MUD, 26 MMRD) after a myeloablative conditioning. During a median follow-up (FU) of 6 months (2-26), WT1 in BM was quantified using real-time PCR (RQ-PCR), with TaqMan technology on RNA from mononucleated cells. The housekeeping gene ABL was used as control gene, with WT1 level being normalized to 10e4 copies of ABL per sample. WT1 expression was monitored considering as MRD negative values < 180 WT1 per 10e4 ABL.
Results: In 19 pts out of 57 (33%) an hematological relapse occurred. At day + 30 after HSCT all 19 pts were in CR, 8 pts with BM WT1 < 180 and 11 with BM WT1 > 180. 10 pts out of 19 (53%) showed an increase of BM WT1 levels above 180 at day +160 (median, range 60-710) after HSCT, 40 days (median, range 20-70) before overt relapse; at the same time-point, we documented hematologic and cytogenetic CR and chimerism on STR analysis showed 100-95% donor component. In 6 pts out of 19 (32%) BM WT1 was <180 105 days (median, range 80-130) before relapse; unfortunately, this group skipped 1 or 2 point of FU for medical decision. 3 pts out of 19 (15%) relapsed within 50 days post HSCT and are not informative as only the day 30 BM evaluation was performed.
Conclusions: post HSCT kinetics of WT1 in BM can predict relapse in AML/MDS pts. A quantitative increase above the threshold of MRD positivity was detected about one month

before hematological AML/MDS relapse, in our pts. Our results suggest that monthly quantitative monitoring of BM WT1 with RQ-PCR is useful to detect early relapse and to drive proper post transplant immunotherapy (e.g. immunosuppression withdrawal or donor lymphocyte infusion) and induce an anti-leukemia effect at an early stage.

P722

Persistence of BCR-ABL1 gene signal in 10-year survivors of myeloablative allogeneic haematopoietic stem cell transplantation for chronic myeloid leukaemia

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Objectives: Allogeneic hematopoietic stem cell transplantation (HSCT) has been the only curative therapy for patients with chronic myeloid leukemia (CML) until the advent of tyrosine kinase inhibitors. Even if studies on the long-term outcome of HSCT for CML have shown very rare relapses after 10 years from transplant, a late molecular assessment of the disease has never been carried out. With the aim of verifying whether patients who survive for many years after an HSCT, apparently free of leukemia, may have residual leukemia cells, we performed a molecular assessment of the BCR-ABL1 gene in all patients transplanted at our Institution and with a greater than 10 years follow-up from transplant.
Methods: Since January 2010 we performed a quantitative real-time polymerase chain reaction (RQ-PCR) analysis, from peripheral blood, in surviving patients who had received a myeloablative HSCT from an HLA identical sibling donor for CML between 1984 and 1999.
Results: Overall, out of 51 surviving patients, 8 refused to participate in the study, 5 were not traceable and 4 patients had developed an hematological relapse. The RQ-PCR analysis was thus performed on 34 patients, with a median follow-up from transplant of 220 months (range 127-327), and in continuous hematological remission lasting more 10 years with a negative cytogenetic analysis monitored during the first 5 years after transplant. RQ-PCR resulted positive in 5 patients (14.7%) with BCR-ABL/ABL IS % 0.2, 0.14, 0.4, 0.7 and 62, respectively. Four patients were in remission 12, 19, 20 and 21 years after transplant, respectively, while 1 patient had had an hematological relapse 24 months after transplant but had obtained a second 16 year cytogenetic remission following donor lymphocyte infusion. The patient at 21 years after transplant, showing 62 IS, concomitantly presented a previously undetected increased number of platelets and white blood cells (CML hematological relapse). The 4 patients with low number of copies and normal blood cell count were monitored with qRT-PCR every 3 months: molecular evidence of disease persisted in 3 cases and disappeared in 1.
Conclusions: Our study shows that residual leukemia cells, identified by molecular analysis, may be found in CML patients long after an allogeneic HSCT. This phenomenon is of unknown prognostic significance, but molecular surveillance in long-term survivors may answer questions regarding patterns of late relapse.

P723

Influenza A virus (H1N1)v in allogeneic haematopoietic stem cell transplant recipients: comparison of the antibody response to monovalent influenza A (H1N1)v vaccine versus the response in natural infection

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Background: Vaccination against influenza seems to be less effective in hematopoietic stem cell transplant (HSCT) recipients than in the immunocompetent population. In addition, little is known about the antibody response in natural infection. The presumed lack of background antibody against influenza A (H1N1)v-virus was an opportunity to compare the antibody response to illness with that observed after vaccination.
Methods: This study, conducted by the Société Française de Greffe de Moelle et de Thérapie Cellulaire, included 90 allogeneic HSCT recipients aged from 18 to 65 years. Patients were either vaccinated with 2, 3-week interval doses of monovalent influenza A (H1N1)v vaccine (GrI), or had a influenza A (H1N1)v illness proved by positive PCR (GrII). H1N1v-specific antibody titers were measured by a hemagglutination-inhibition assay, at days 0, 21 and 42 after the first vaccine dose in GrI patients (pts), and in both frozen pre-pandemic and post-infection serum in GrII.
Results: 70 pts were included in GrI and 20 in GrII. In GrI, 59 pts received an adjuvanted vaccine (GrIAdj+) and 11 a non-adjuvanted vaccine (GrIAdj-). There were no significant differences in patients' characteristics according to the group (sex, type of conditioning regimen, source of stem cells, type of donor, incidence of acute- or chronic graft-versus-host disease (GVHD)). GrII pts were younger. The time interval between HSCT and infection was longer than between HSCT and vaccination. None of the vaccinated patients presented an (H1N1)v-virus disease. The percentage of seroprotection (1/40) at day 42 after vaccine, or post infection, was similar in the 3 groups: 66%, 50% and 60% in GrIAdj+, GrIAdj- and GrII respectively (p=0.59). The ratio of geometric mean titers (RGMT) at day 42/day 0 of vaccination or pre/post infection were 13.6, 3 and 9.1 in GrIAdj +, GrIAdj- and GrII respectively, with a significant difference between GrIAdj+ and GrIAdj- (0.04). In the multivariate analysis, 3 factors were associated with higher rate of post-infection/ second vaccine seroprotection: a longer time interval between HSCT and vaccination/ infection, the absence of chronic GVHD and a myeloablative conditioning regimen ($p<0.05$). RGMT were correlated with the same factors ($p<0.05$).
Conclusion: 1) More than half of the HSCT recipients reach a seroprotection after infection. 2) Two doses of vaccine induce a humoral response similar to that observed after natural infection in HSCT recipients.

P724

Mesenchymal stromal cell co-infusions increase early lymphocyte recovery and T-cell reconstitution in autologous haematopoietic stem cell transplantation

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Early lymphocyte recovery (ELR) after autologous hematopoietic stem cell transplantation (AHSCT) predicts better survival in patients with hematological malignancies. We have previously showed the co-transplantation of low doses of autologous

bone-marrow derived mesenchymal stromal cells (MSCs) was safe and resulted in improved granulocyte and platelet recovery in lymphoma patients. The purpose of the present study was to evaluate whether co-infusions of MSCs influence early lymphocyte recovery and T cell reconstitution. 126 patients with Hodgkin`s lymphoma (46), non-Hodgkin`s lymphoma (52) and multiple myeloma (28) who underwent AHSCT at the Department of Hematology and Bone Marrow Transplantation of Institute of Clinical Immunology were enrolled in this investigation. Among them 65 patients were co-transplanted with MSCs (MSC+) and 61 patients underwent standard AHSCT without MSC co-infusions (MSC–). MSC+ and MSC– groups were related by age, gender, diagnosis, previous treatment, mobilization and conditioning regimens and dose of transplanted CD34+ cells. Mean dose of transplanted MSCs was $0.16x10^6$/kg. Patients given MSCs showed significantly higher frequency of ELR (absolute lymphocyte count ≥500 cells/μl at day 15 following AHSCT) compared with controls (71 vs 43%; P<0.004). Besides, they did not experience increase of infection rate and revealed enhancement of relative and absolute number of naïve T helper cells (CD4+CD45RA+) (P=0.026) by the end of the first post-transplant month, evidencing better CD4 reconstitution. MSC effect was more prominent in patients with lower doses of CD34+ cells (<4.4 x 10^6/kg) (P=0.02) and did not dependent on absolute lymphocyte count in apheresis product. These data suggest that low doses of MSCs do not possess immunosuppressive effect and may improve lymphocyte recovery and T-cell reconstitution following AHSCT.

P725

Interest of negative minimal residual disease estimated by flow cytofluorometry after allogeneic stem cell transplantation for chronic lymphocytic leukaemia

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Introduction: Minimal residual disease (MRD) eradication in patients treated by standard therapies for chronic lymphocytic leukemia (CLL) correlates with improved outcome. However there is limited information about the interest of negative MRD after allogeneic stem cell transplantation (allo-SCT). We investigated whether blood phenotypic remission could impact post-transplant outcome in patients with CLL.

Methods: We retrospectively included 33 patients who underwent allo-SCT for CLL and with post-transplant MRD monitored by 4 or 6-colour flow cytofluorometry in blood samples. Prognostic impact was evaluated on overall survival (OS) and progression-free survival (PFS) according to the best response and 12 month-MRD status.

Results: Median age at transplant was 54 years (41-66). Status at transplant was available in 27 patients: 11% had negative MRD, 26% haematological complete response (CR), 59% partial response (PR) and 4% refractory disease. Median number of MRD evaluations after transplant was 5 (1-23). Response to transplant: After transplant, 16 patients achieved negative MRD, 15 haematological CR, 1 PR and 1 did not respond to transplant. Among the 16 patients with phenotypic CR, negativation of MDR was obtained before the cessation of immunosuppressive therapy for 15 of them. Median time to negativation was 7 months (2-20). In patients achieving phenotypic remission chronic GVHD rate was 75% versus 44% in the others. Post-transplant outcomes: With a median follow-up of 27 months, the 2-y OS and the 2-y PFS were respectively 84% and 53%. Cause of death (n=8) was progression in 4 cases and transplant related mortality in 4 cases. Impact of phenotypic CR: 2-y PFS was 85% in patients achieving phenotypic CR, whatever the time of evaluation, versus 27% in the others (p=0.012). 19 patients had MRD evaluation available at 12 months after transplant: 2-y PFS was 100% in 12-month-negative-MRD patients (n=10) versus 17% in positive-MRD patients (p=0.003). No relapse was observed in patients who achieved phenotypic remission at 12 months post-transplant.

Conclusion: These data suggest that achievement of post transplant negative MRD in patients with CLL is associated with a long-term control of the disease and better PFS. In our series, no relapse occurred in patients with negative MRD at 12 months post transplant. These results could lead to decrease immunotherapy and administrate donor lymphocytes to patients with post-transplant persistent positive MRD.

P726

Responses to DLI, in the context of pre-DLI lymphoreduction, may be predicted by higher absolute T regulatory cell counts and resistance of CD8 central memory cells to killing by fludarabine

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There are few factors that consistently predict responses to Donor leukocyte infusions (DLI) for mixed chimerism (MC). Based on our previous evidence that patients with a lower peripheral blood lymphocyte count were more likely to respond, we instituted a clinical trial using lymphoreduction pre-DLI, in patients with a high lymphocyte count, to improve overall responsiveness. We postulated that there may be higher numbers of Tregs when the lymphocyte count was high, which may suppress the infused T cells.

Patients received three doses of oral fludarabine on day -7, -6 and -5. Samples were taken at D-7, D0, D+7, D+28, D+60 and D90. Samples were analysed for a combination of markers for CD4, CD8 and T regs using multicolour flow cytometry. CD4 and CD8 were also analysed for subsets-central memory (CM 62L+RA-), effector memory (EM 62L-RA-), naïve (N 62L+RA+) and effectors (EFF 62L-RA+). T regs were characterised as CD4+25hi127lo.

Of the 14 patients analysed to date, 10 (71%) responded to a single dose of DLI while 4 were non-responders. There was a significant decrease in the absolute numbers of CD8 (unpaired t test, p<0.001) and CD4 (unpaired t test, p<0.001) between day -7 and D0 for all patients. The absolute number of T regs was not significantly changed. Contrary to our hypothesis, DLI responders showed a significantly higher absolute T reg count at all time points, compared to non-responders (unpaired t test, p<0.004). There were no significant differences in CD4 or CD8 counts. In addition, DLI responders showed a significantly higher T reg/CD8 ratio of means compared to non-responders at all time points (unpaired t test p<0.04). CD8 subset analysis showed a significant reduction in the CD8 CM cells in the responders between D-7 and D0, compared to non-responders (unpaired t test, p<0.001). Non-responders showed higher absolute CD8 CM counts at all time points compared to responders trending towards significance (unpaired t test, p=0.054). Other CD8 subsets and CD4 subsets did not show any significant difference.

In conclusion, lymphoreduction was a successful strategy resulting in good responses. There appeared to be persistence of the CD8 CM cells in the non-responders. Interestingly, there were lower absolute count of T regs in the non- responders suggesting that these patient may require an alternative strategy as the levels are not improved by fludarabine. Randomised studies with robust sample analysis are required to resolve these mechanisms.

P727

Rapid transition of CD4 naïve cells to effector/memory phenotype and low expression of NK receptors: early results from the Immune Reconstitution Post Cord Blood Transplantation (IRES-CBT) UK national trial

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Delayed engraftment and infections remain a significant problem following unrelated cord blood transplantation (UCBT). One cause for delayed immune reconstitution (IR) is the use of serotherapy (ATG) in the conditioning regime. NK cells play an important role in innate immunity and are the predominant cell subset to appear in the early post transplant period. NK cell cytotoxicity depends upon of activation of receptors such as DNAM-1, 2B4, NKG2D and NKp46. These receptors play a vital role in clearing virus infected cells and in tumour surveillance.
As part of a national UK UCBT trial we aim to investigate the IR obtained in the absence of serotherapy. Peripheral blood samples are collected at D28, D60, D100 and 6months post transplantation. Samples were tested for a combination of markers using multicolour Flow cytometry for T, B, NK and their subsets, monocytes and DC's.
Thus far 6 patients have been studied (median age 42 (21-57)). Patients were transplanted for high risk/relapsed leukemia (4), Hodgkins disease (1) and T cell lymphoma (1) using reduced intensity conditioning (RIC) following the Minnesota protocol. HLA matching was at least 4/6 for low resolution HLA-A, HLA-B and high resolution for HLA-DRB1. Ciclosporin and mycophenolate mofetil was used as GvHD prophylaxis.
The results so far show that there is a gradual increase in all mononuclear subsets- T cells, B cells, NK cells and monocytes from D28 to 6 months, but the values still remained lower than normal. Naïve B cells were the predominant B cell subset. There is a rapid expansion of CD4 T cells compared to CD8 T cells with a rapid transition from naïve CD4 to effector/memory phenotype at D60. CD8 cells were predominantly effector/memory from D28 onwards and CD56dim NK cells were the predominant NK cell subset. Expression of NK receptors NKG2D, NKp46, 2B4 and DNAM-1 was lower than healthy controls (adult and cord), particularly for NKG2D and DNAM-1 (at D60 and D100 respectively).
These early results suggest that unrelated CBT without serotherapy appears to be associated with a faster reconstitution of T cells and the development of CD4 and CD8 memory cells. Despite the rapid reconstitution of NK cells, the expression of NK receptors remained low (D100). More patient samples and clinical correlates will help us to clarify the functional impact of these findings on the immune reconstitution pattern observed following UCB transplantation.

P728

Immunotherapy based on serial and quantitative lineage specific chimerism following allogeneic peripheral blood stem cell transplantation

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Objective: To find out the relationship between chimerism and relapse after allogeneic hematopoietic stem cell transplantation (allo-HSCT) and to evaluate individualized immunosuppressant therapy and donor lymphocyte infusion (DLI) based on serial and quantitative chimerism.
Methods: T lymphocyte, B lymphocyte and NK cell chimerism were detected by PCR amplification of short tandem repeats in 113 successfully engrafted patients after allo-HSCT. Individualized immunosuppressant therapy and DLI were given to patients according to chimerism.
Results: 17 patients had decreasing chimerism, but T cell remained MC on day 30 and/or 60 posttransplant. All of them reached FDC after their immunosuppressant was decreased by 25% to 50%, Only 4 patients developed de novo acute graft-versus-host disease (GvHD) after the modulation. 14 patients undergone dosage reduction or even cessation of immunosuppressant when they had increasing MC by 5% to 80% in T lymphocyte on day 21 to 150 posttransplant, None of them had disease recurrence. 29 patients (25.66%) relapsed or progressed after median 151 days posttransplant. 13 patients with acute or chronic myelogenous leukemia had increasing MC in T lymphocyte by median 4.8%, increasing MC in NK cells by 34.5% when disease recurrence; 11 patients with acute B lymphoblastic leukemia had increasing MC in T lymphocyte by median 18.2%, increasing MC in NK cell by median 15.0% and increasing MC in B lymphocyte by median 59%. All were given cessation of immunosuppressant and 13 of them were given dose escalating DLI after relapse or progression. 3 of them achieved complete remission again, the others died of disease relapse. Acute grade ≥ GVHD occurred in 32.83% in 67 patients who remained stable FDC. In the remaining 46 patients who received immunosuppressant dosage change because of persistent MC or increasing MC, 32.61% developed grade ≥ II GVHD. The chronic GVHD incidence was 62.83% and 61.19% respectively in these two groups. There was no significant difference in the incidence of both acute grade ≥ II GVHD (P=0.924) and the chronic GVHD (P=0.315) between the two groups.
Conclusions: T, B and NK lymphocyte chimerism could be used as relapse predictor of hematological malignancy. Individualized immunotherapy based on chimerism could delay or avoid clinical relapse, but did not increase the incidence of acute or chronic GVHD.

P729

Disease-specific recovery of regulatory T-cell after allogeneic stem cell transplantation

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Allogenic stem cell transplantation (allo SCT) offers a potential curative option for many malignant and non malignant haematological diseases. Despite its therapeutic benefit, immunodeficiency, poor immune reconstitution and Graft vs. Host disease (GvHD) can often be limiting drawbacks. Regulatory T cell subsets (Treg) have been described and several lines of evidence indicated their implication on GvHD occurrence and progression. We analysed the Treg reconstitution of 184 patients who underwent allo SCT at our Center from 2007 till 2009.
Differential lymphocyte subsets were analysed by flow cytometry. Tregs were evaluated on simultaneous expression of CD4/CD25hi/CD127low. Data were obtained in monthly interval for the first 6 months and thereafter every 6 months for the next 3 years. Data were analysed for the three different subgroups: Multiple Myeloma (MM: n=83), Myelofibrosis (PMF: n=22) and AML/MDS (n=51).
The mean value of Treg cell number before allo SCT was 2,5% of the total leukocyte number in all patients. There was no significant difference in the Treg level in any of the three major groups. All patients exhibited a significant reduced number of Tregs during the first 30 days after allo SCT (MM: 0,79%; p=0,009; PMF: 0,41%; p=0,01; MDS/AML: 0,6%; p=0,01). Between day 30 and 60 after allo SCT patients with MM had a transient Treg recovery to baseline level while Treg of patients with PMF or MDS/AML remained significantly lower in comparison to baseline value (PMF: 0,72%, p= 0,002 and MDS/AML 0,81%, p=0,01 respectively). One year after allo SCT a Treg recovery (1,3% and 1,8% respectively) was observed in MM and MDS/AML patients while PMF patients

still maintained a significant reduction (0,65%; p=0,01). Interestingly, 2 years after allo SCT, Treg levels were decreased in all 3 subgroups (MM: 1,1%, p=0,008; PMF: 0,7%, p=0,02 and MDS/AML: 0,7%, p<0,0001), while after 3 years Treg counts achieved pretransplant level. In contrast to Treg, total T cells are only transiently but significantly reduced within the first 180 days.
A highly dynamic Treg recovery after allo SCT was observed in our group of patients. Our data highlight that there is a difference in Treg recovery among the various fore mentioned diseases. Treg reconstitution appeared to be prolonged in patients with PMF in comparison to the other subgroups. Our data provide a basis for further analysis towards differential Treg reconstitution and its potential impact on allo SCT complications.

P730

Cytogenetic risk groups and minimal residual disease are strong prognostic factors for post-transplant outcome in patients with acute myeloid leukaemia

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Allogeneic stem cell transplantation (alloSCT) is a curative treatment option for patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). Relapse after alloSCT is still a major cause for the treatment failure. Molecular genetic (FLT3, NPM1 mutations) and cytogenetic risk-categories have an important impact on the prognosis of patients undergoing alloSCT. However, it has been shown that there is a closely relationship between the level of minimal residual disease (MRD) and relapse in leukemia patients.
We analyzed 140 AML/MDS patients transplanted at our institution. We examined the contribution of cytogenetic aberrations and molecular genetic markers detected prior alloSCT to survival, relapse, and mortality after transplantation. Furthermore, we analyzed MRD status after consolidation therapy and in post-transplant period. We classified genetic/cytogenetic status before alloSCT into 2 groups: Low-risk: good and intermediate-risk karyotype, FLT3-wt, NPM1-mutated cases and High-risk: adverse-risk cytogenetic, FLT3-ITD mutated cases. A good risk karyotype was present in 8 patients; an intermediate risk in 56 patients, whereas 76 patients had a poor cytogenetic risk. 51 patients were treated with standard myeloablative conditioning regiments prior to alloSCT, 73 patients received reduced intensity conditioning and 16 non-myeloablative conditioning. 40 patients had a matched-related donor and 100 patients had a matched-unrelated donor. Overall survival (OS) in the group of patients with Low cytogenetic risk was 839 days as compared with 613 days in High-risk group. The Low risk cohort also showed a lower relapse (33% vs. 51%, p<0,03) and mortality rate (20% vs. 71%, p<0,003). We analyzed MRD status in all AML patients after consolidation therapy. MRD negative patients are characterized by favorable prognosis as compared with MRD positive patients, OS 87% vs. 53%, p<0,001. We conclude that risk-stratification combining molecular genetics and cytogenetics aberrations at the time of diagnosis with post-consolidation MRD status improve identification of high-risk category of patients.

P731

Early immune reconstitution after haplo-identical PBSCT with RIC and use of non-T-depleted graft in children with cancer

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Delayed immune reconstitution and vulnerability to infection supposed to be the main obstacles to successful stem cell transplantation from alternative donors, especially after the procedure of graft selection/depletion and use of anti-thymocyte globulin.
We measured the amount of T- B- and NK- cells after haplo-PBSCT with reduced intensity conditioning regimen (RIC) in 17 children with advanced malignancies. Horse ATG was included in regimen. The only graft manipulation was in vitro incubation with vincristine and methylprednisone. All pts in this group grafted successfully at an average d+11. We didn't see any dramatic decrease in CD3+ or CD4+cell count early after engraftment and later on. The mean CD3+ at the d+30, d+100 and +6mo were: 0.9, 1.5 and 1.6x10^9/l respectively. The mean CD4+ at the same time points: 0.4, 0.6, 0.4x10^9/l, this may be due to prolonged immunosuppressive treatment in such category of pts. Early recovery of CD56+ (0.3x10^9/l at d+30) was important for residual disease control. The mean CD19+ early after transplantation was about 0.06x10^9/l and returned to normal range by +6mo. Only 18% pts had acute GVHD III; there were no cases of acute GVHD IV. Infection was mild.
Thus RIC and use of non-T-depleted graft enabled rapid immune reconstitution and early tumor control opportunity after haplo-SCT at the time when GVHD was manageable.

P732

Chimerism kinetics of unfractionated bone marrow in the first year after allogeneic haematopoietic cell transplantation is predictive of relapse in patients with AML and MDS

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Prediction of relapse after allogeneic hematopoietic cell transplantation (allo-HCT) by detection of minimal residual disease is not always feasible, especially in AML and MDS. As the predictive value of monitoring chimerism remains unresolved, we retrospectively explored whether monitoring chimerism of unfractionated bone marrow (UBM) samples can be predictive of impending relapse. The study group included 68 patients who underwent allo-HCT for (i) AML (65) and, (ii) MDS (3) followed-up for 31 (1-98) months. The chimerism was assessed by genotyping short tandem repeat polymorphisms (STRs) of the VWA, FES, THO1, SE33 and F13A1 genes or by determination of allelic variants of the AMEL genes. The monitoring was performed at days +14, +30, +60, +90 post-transplantation and thereafter every 3 months for up to 2 years. Patients were stratified as having complete chimerism (CC), decreasing recipient mixed chimerism (DMC), increasing recipient mixed chimerism (IMC), increasing and decreasing mixed chimerism (MC) and stable mixed chimerism (SMC) at regular time intervals (0-3, 3-6, 6-9, 9-12, 12-18, 18-24 months). Patients with a <5% increase of autologous hematopoiesis at only one event were stratified as having low level mixed chimerism (LLMC). CC/DMC/LLMC and IMC/SMC/MC patients were classified as presenting favorable or unfavorable chimerism kinetics (FCK or UCK), respectively. A total of 894 UBM samples were analyzed. CC at all time points was documented in 15/68 (22%) patients, all treated by myeloablative conditioning. The cumulative incidence of relapse was 29% (20/68 cases). Patients with FCK at 3 month intervals between 3 and 12 months were less likely to relapse, presented a longer overall survival (OS) and were more likely to develop cGvHD (p<0,05 for all comparisons). Closer monitoring during the first 6 months showed that patients with overall FCK were less likely to relapse (9/44, 20%, vs 11/24, 46%) and more likely to develop cGvHD (33/44, 75% vs 14/24, 58%). Chimerism kinetics during the first year remained a significant predictor for both OS and relapse in a multivariate model, while incomplete chimerism at days +14 and +30 were not clinically relevant. Our data suggest that assessment of chimerism during the first year and not the second post transplantation may predict imminent relapse and guide early intervention. A closer monitoring may be justified for high-risk patients with stable or increased mixed chimerism.

P733

Long-term persistence of functionally altered GPI anchor negative memory T-cells after alemtuzumab-based T-cell depleted haematopoietic stem cell transplantation

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The anti-CD52 antibody alemtuzumab is frequently used for in vivo T cell depletion (TCD) in the context of allogeneic hematopoietic stem cell transplantation (HSCT). We have recently demonstrated the persistence of CD52neg T-cell subsets in patients after HSCT following alemtuzumab-mediated TCD. Without the addition of donor lymphocyte infusions (DLI), CD52neg T cells were detected in significant proportions (up to 40%) even more than 3 years after HSCT in our patients. The impaired expression of CD52 is caused by a loss of glycosylphosphatidyl-inositol- (GPI) anchors on the cell surface and is associated with the lack of further GPI-anchored molecules.
When we analyzed the antigen-specific T-cell response by ELISPOT assay and cytokine-secretion assay following stimulation with CMV-peptide loaded autologous dendritic cells, we found an impaired IFN-γ secretion of GPI-anchor negative T cells. In addition, they showed decreased lytic capacity of CMV-infected fibroblasts compared to GPI-anchor positive T cells. Here we also investigated on different T-cell subpopulations with regard to their GPI-anchor expression. We analyzed the peripheral blood T cells of 10 patients with different age obtained in the course after HSCT by flow-cytometry. To differentiate naïve and memory T cells, we stained for expression of CD45RA, CD45RO, CD62L and CCR7. GPI-anchors were stained directly using FLAER (fluorescent aerolysin).
GPI-anchor positive and negative populations were detected among memory CD4 as well as memory CD8 T cells. Early after transplantation no naïve T cells were found. Beyond the first year after transplantation, CD45RA/CD62L/CCR7pos T cells were again detected in some of our patients. These naïve T cells were regularly GPI-anchor positive. Our data are in line with the hypothesis that graft-derived GPI-anchor negative T cells outgrow in the presence of alemtuzumab after HSCT and persist beyond the selective pressure of the antibody. However, newly generated naïve T cells as well as DLI derived memory T cells express GPI-anchors at normal levels.
In summary, we propose that the functionally impaired GPI-anchor negative T cells are memory T cells. Some patients with preserved thymic function, e.g. younger patients, might restore GPI-anchor positive naive T cells later after HSCT. The predominance of GPI-anchor negative memory T cells with impaired antiviral reactivity could be one reason for the increased viral infections after alemtuzumab-related TCD.

P734

Evolution of mixed chimerism after allogeneic haematopoietic stem cell transplantation in patients with haematologic malignancies

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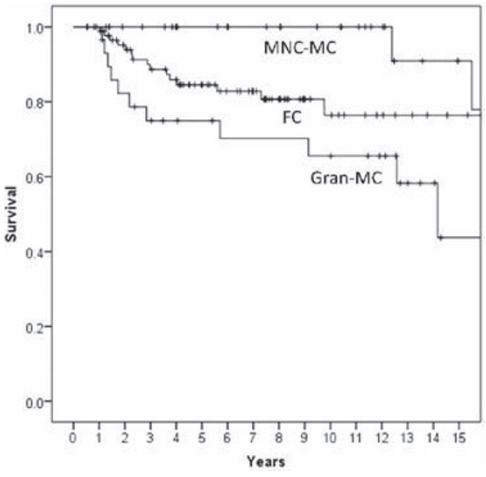
Allogeneic hematopoietic stem cell transplants (HSCT) may result in mixed chimerism (MC), more frequently, when grafts are T-cell depleted. Increasing levels of MC may indicate disease relapse or impending graft rejection. MC may be stable over time and compatible with prolonged remission. We analyzed in this observational study the evolution of chimerism in allotransplanted patients with hematologic malignancies, alive without relapse at 1 year post HSCT, and compared the outcomes of patients with “lineage restricted” MC (granulocytes vs mononuclear cells chimerism) with those with full donor chimerism (FC).
Patients and methods: We retrospectively analyzed data of all patients, transplanted between 1986 and 2006, for hematologic malignancy, alive without relapse at 1 year post HSCT. Transplants were T-cell depleted in patients with low relapse risks, but not in high risk disease. Chimerism was tested using short tandem repeat polymorphisms after separation into mononuclear cells (reflecting lymphocytes and monocytes) and granulocytes by Ficoll density gradient centrifugation.
Results: Of 155 patients studied, 89 had FC, 36 mononuclear cells MC (MNC-MC) and 30 granulocytic with or without mononuclear cells MC (Gran-MC). There was no differences for age, sex, disease, conditioning, donor type, stem cell source and donor lymphocyte infusions between the 3 groups ($p>0.05$), but there was more MC in patients with T-cell depletion ($p< 0.001$), and less MC in patients with acute graft-vs-host disease (GvHD) ($p=0.045$), chronic GvHD ($p=0.009$) and female donor into male recipient transplants ($p=0.001$). Survival was significantly better in MNC-MC than in Gran-MC patients ($p=0.001$), with FC patients being intermediate (see Figure). There was more disease relapse in the Gran-MC group but not in the MNC-MC group as compared to FC ($p=0.026$). MC was stable over prolonged periods in some patients in the Mono-MC and the Gran-MC groups (up to 21 respectively 19 years). Of MC patients alive at 10 years, MC persisted in 83% (15/18) in the MNC-MC and 57% (8/14) in the Gran-MC groups.
Conclusion: Mixed chimerism may remain stable over a long time period. In survivors without relapse at 1 year post HSCT, determining lineage specific chimerism may be useful as outcome differs, MNC-MC being associated with better outcome than Gran-MC. MNC-MC probably reflects persistent recipient lymphopoiesis which is not associated with increased relapse risk.



P735

A case report of persistent mixed full donor chimerism in different cell lineage after double unit cord blood transplantation

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Double-unit cord blood transplantation (CBT) has been established as an alternative source of donor cells for allogeneic haematopoietic stem cell transplantation (HSCT). We reported here a case of long-term mixed full donor chimerism during the regular follow-up of three years after HSCT.
A 23-year-old woman with acute lymphoblastic leukaemia in second complete remission received two units of cord blood (D1+D2) after a myeloablative conditioning regimen. The transplanted units were 4/6 HLA-A,B antigen and DRB1

allele matched to the recipient. The infused total nuclear cell (TNC) of the two units were respectively 1.8x10^7 and 1x10^7/kg, whereas the CD34 cells number was identical in the two UCBs and the CD3 cells number was twice in D1 (table 1). Neutrophil recovery was observed at day 34 and platelets engraftment at day 55 after CBT. Only one event of acute graft-versus-host disease grade I was reported at day 49. Nowadays, the patient has no complications. Analysis of chimerism was performed by STR-PCR or RQ-PCR on whole blood and specific lineage cells (CD3, CD15 and CD19). Full donor chimerism was achieved on day 60. Each of the two units contributed at different levels to the donor chimerism in specific lineage cells: whole blood and CD15 were about 50% D1/D2, CD19 cells were preferentially from D2 origin (65%), and CD3 cells were from D1 origin (75%). Tolerance between cells issued from two different UCBs is shown by a persistent full mixed chimerism after 19 months of follow-up and it was confirmed after 36 months. Kir ligand analysis showed an absence of mismatch in GvH direction between recipient and each UCB while there was a mismatch between the 2 units. Tolerance of both cords was observed following the mixed lymphocyte reaction. Selection of CD4 specific cells of two UCBs was performed on whole blood recipient based on HLA-A2 antigen, and cells were mixed together in both direction in tissue culture. Indeed, alloreactivity of D2 seems to be more important than D1, which would be in “anergy” (Figure 1). In this case, a state of full tolerance settled down between the various lineages, either immune mediated interaction between host/graft or between graft/graft could explain this chimerism pattern, but it will have to be clarified by a specific study of Treg cells.
The mechanism involve in tolerance is still open between anergy or regulation by a third party cells. Immunology department was deepened this part of the study.

	HLA-A	HLA-B	HLA-DRB1	TNC 10^7/kg	CD34 10^5/kg	CD3 10^7/kg	Blood group
Recipient	0101 6802	2703 3801	0404 1401	-	-	-	O+
Donor 1	01 68	48 55	0404 1401	1.8	0.7	8.1	O+
Donor 2	02 68	27 44	0404 1401	1	0.7	4.4	O+

Table 1: Characteristics of the 23-year-old woman and the two UCBs. Two and four digit resolution HLA typing is given for the patient and the donors. Number of transplanted cells were calculated per kg bodyweight.

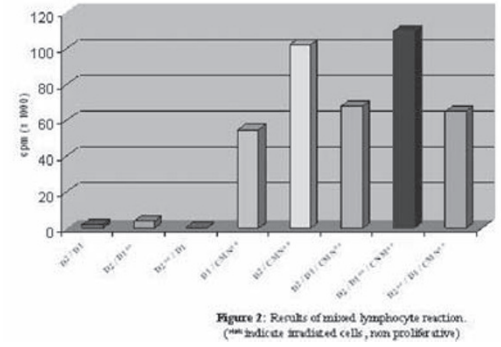

Figure 2: Results of mixed lymphocyte reaction. (* indicate irradiated cells, non proliferative)

P736

Long-term immune reconstitution in HIV-positive relapse/refractory lymphoma patients after autologous stem cell transplantation

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Objectives: To assess immune recovery in long term survivors relapse/refractory HIV-positive (HIV+) lymphoma patients (pts) submitted to Autologous Stem Cell Transplantation (ASCT). To correlate immune recovery with virological and clinical parameters.
Methods: HIV+ pts who underwent ASCT at the National Cancer Institute (Aviano, Italy) and with at least 60 months (mo) of follow-up after transplantation were included in this retrospective immunological study. CD4, CD8 absolute T lymphocyte subset counts were evaluated by flow cytometry (EPICS XL–Beckman-Coulter). Thymic regeneration was evaluated by assessing the number of sjTRECs per 10^6 peripheral blood mononuclear cells (PBMCs) using a real-time polymerase chain reaction (PCR) quantitative technique. The HIV RNA level was quantified by using the Versant HIV-1 RNA 3.0 assay kit (bDNA; Bayer Diagnostics).
Results: 10 relapse/refractory HIV+ lymphoma pts were studied: 8 had sixty, 7 seventy-two, 3 eighty-four and 3 ninety-six mo follow-up from ASCT. Pre-transplant median CD4 T lymphocyte levels were 191 cell/microL (range: 13-460), a significant increase was observed by mo 24 after ASCT (median 300 cell/microL, range: 87-628, p=0.04) and at least a doubling of all the pre-transplant values at mo 72 (median 586 cell/microL, range: 379-1028). Six pts reached CD4 T cell levels within a normal laboratory range (480-1315 cell/microL) during a median follow-up of 36 mo (range: 24-72), 50% being admitted to salvage therapy with CD4 T cell levels below 200 cell/microL. CD8 T lymphocyte counts were expanded in the majority of the pts, and ratio of CD4 cells to CD8 appeared below 1 for the entire follow-up, except in 2 pts. sjTRECs/10^6 PBMCs values increased significantly between pre- and post-ASCT (p<0.01) and in 2 pts reached the normal range correlated to the respective age group at mo 24 and 48. All pts were pre-treated with HAART based on their clinical antiretroviral therapy histories and/or the HIV genotypic test result for a median of 38.6 mo (range: 8-86) before enrolment. For 4 pts HAART was changed during ASCT protocol to re-establish its best efficacy. All but 1 pt showed HIV RNA levels below the detection limit (50 copies/mL) during the long term follow-up post-ASCT.
Conclusions: relapse/refractory HIV+ lymphoma pts with long term follow-up post ASCT show a very good immune and thymic function recovery. Response to HAART is important, but, perhaps, not the unique favourable determinant.

P737

Y-chromosome-specific real-time PCR approach improves sensitivity of chimerism analysis in paediatric patients with haematological malignancies given stem cell transplantation

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Donor/recipient mixed chimerism (MC) may be associated with an increased risk of disease recurrence in patients with haematological malignancies given haematopoietic stem cell transplantation (HSCT). Early detection of MC is essential to design therapeutic strategies to control/prevent leukemia relapse. MC is mainly assessed by analysing short tandem repeats (STRs) and, when the patient is male and the donor female, Y-chromosome-based amelogenin locus (yPCR), with a sensitivity of 4-5% and 1-2%, respectively. The emerging technology of quantitative real-time PCR (qPCR) could potentially offer the greatest sensitivity, especially using Y-chromosome markers. Aims of this study were a) to evaluate donor/recipient chimerism, comparing the results obtained by a Y-chromosome specific qPCR with those obtained by yPCR in terms of sensitivity, c) to correlate chimerism status with clinical outcome in male children with acute leukemia given HSCT from a female donor. We analyzed 197 samples, obtained from bone marrow (BM, n=91) and peripheral blood (PB, n=106) of 28 patients at different time points after engraftment. In 118 out of 197 samples

MC was detected by qPCR, whereas yPCR allowed the detection of MC in 51/197 samples. Twenty-three of the 67 samples in MC only by qPCR were from two patients, who relapsed 3 and 5 months after transplant respectively. Patients were analyzed weekly. The first exhibited MC 2 and 1 months before clinical evidence of recurrence by q- and yPCR, respectively and the second showed MC by yPCR at time of clinical relapse, whereas qPCR allowed the detection of progressive MC starting from 3 months before recurrence. Forty-four samples from 9 children showed very low levels of MC in PB within a median time of 6 months after transplant, then converted to FDC. To improve our understanding of the significance of MC, we investigated the possible reappearance of host haematopoietic cells in BM cell subsets selected according to immunophenotype of the leukemia blasts at diagnosis and in PB T-, B-, and NK-lymphocyte subsets: all BM samples showed FDC, whereas MC was detected particularly in T-lymphocytes.
These results suggest that chimerism analysis assessed by qPCR using Y-chromosome marker allow the detection of MC earlier compared to yPCR, when the number of host cells is very low. qPCR represents a useful tool to carry out therapeutic interventions to control leukemia relapse in patients with malignant haematological diseases given HSCT.

P738

Split chimerism may be sufficient for control of immune defects in the presence of CD3+ donor cells

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Background: non malignant diseases may be cured with HSCT. At difference with malignancy, complete eradication of the preexisting hematopoiesis is not mandatory. Thus, to reduce TRM, reduced-intensity regimens have been developed. This led to a higher frequency of mixed chimerism. In this setting, selective engraftment and/or persistence of different subpopulations may occur. Curative effect of HSCT may be exploited by different subpopulations. In patients with congenital cellular immune deficiencies, normal donor lymphocytes play a crucial role in the disease correction.
Study design: retrospective analysis of chimerism and outcome of patients with immune disorders treated with non-fully myeloablative conditioning regimens.
We report 3 cases:

1) 7 month male, SCID T-B+, rIL7, transplanted in emergency while in the Intensive Care Unit, without conditioning regimen, from his mismatched mother. He developed mixed chimerism treated with DLIs; at 3 years from transplant, his CD3+ cells are full donor, while CD3- cells are full recipient.
2) 20 month male, Evans' syndrome, received his HLA-identical brother's BM cells, conditioned with melphalan (140 mg/m2), thiotepa (10 mg/kg), fludarabine (40 mg/m^2/day x 4). He developed mixed chimerism treated with DLIs. At 7 years from transplant his CD3+ cells are full donor, while the CD3- cells are full recipient.
3) 18 month female, Familial Hemophagocytic Lymphohistiocitosys, transplanted with her HLA-identical brother's BM cells, condotioned with Treosulphan (14 gr/m^2 x3), fludarabine (40 mg/m^2/day x4). She developed mixed chimerism treated with DLIs. At 4 months after transplant, her CD3+ cells are 40% donor. All three children are in remission for their diseases.

Conclusion: the use of non myeloablative conditioning regimens allows reduced TRM. Our patients survived their life threatening/fatal disorders and remain disease free for up to several years. Close monitoring of post-transplant chimerism including cell subpopulations confirmed that in these patients with cellular immune defects stable engraftment of CD3+ cells was sufficient to mantain a complete the disease control.

P739

Oral fludarabine as pre-DLI lymphoreduction is well tolerated and effective in converting mixed chimerism to FDC post allogeneic transplant: results from a prospective clinical trial

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Donor leukocyte infusions (DLI) are used following reduced intensity conditioned (RIC) transplants to convert mixed chimerism (MC) to full donor chimerism (FDC). There are few factors which have been consistently shown to predict for responsiveness. We recently reported conversion to FDC rates, after several DLI doses, of approximately 50%. Patients with a high peripheral blood lymphocyte count were significantly less likely to respond. To investigate this novel observation, we instituted a prospective pilot trial (CCR2942). Inclusion criteria were: 1. mixed chimerism in whole blood (< 95%), 2. previous RIC or failure to respond to a previous dose of DLI and 3. a lymphocyte count of >1.0 x 109/l. Patients consenting to the trial received three doses of oral fludarabine as an outpatient at 25mg/m2 on day -7, -6 and -5. To date, 16 patients have been entered onto the trial and 14 have reached the study end point (day 90 chimerism) (1 early death due to leukaemia relapse, 1 currently too early for assessment). 15 received a single dose of DLI and 1 patient had 2 doses. Disease categories were: AML (8), ALL (1), MDS/CMML (3), T-PLL (1), HD (1), DLBCL (1) and MCL (1). 2 patients had co-existing evidence of disease (morphological relapse of AML, 2% positive by immunophenotyping in T-PLL). 10 males, 6 females. 11 sibling donors, 5 unrelated donors. Of the 14 eligible patients, 10 (71.4%) have responded to a single dose of DLI (CR=9, PR=1). Median age: 58 years (range: 22-66). The median time to DLI post-transplant was 7.2 months (range: 3-11). Median percentage of donor chimerism pre-DLI was 85% (range: 18-93). Median lymphocyte count pre-DLI was 1.3 (range: 1.0-2.7). Five patients developed GvHD – grade 1 (liver x2, skin x1), grade III x1 (liver/GI), grade IV x1 (liver/skin). GVHD resolved completely in all cases. 3 patients reactivated a virus (CMV, 2 x EBV). Lower chimerism level pre-DLI showed a trend towards a worse response rate (p=0.095, Fishers exact test), but no other factors were statistically predictive of outcome.
In conclusion, in patients with MC, the use of pre-DLI lymphoreduction results in good response rates, superior to a historical cohort. The incidence of GVHD and adverse events is low. For patients with very low levels of donor chimerism or frank disease relapse an increased intensity of pre-DLI chemotherapy is likely to be necessary. Larger numbers and randomised studies are required to investigate the efficacy of this approach further.

P740

Dynamics of chimerism in activated leukocytes (CD25+) improves anticipation of acute GvHD after HLA-identical related allogeneic stem cell transplantation

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Introduction: Chimerism dynamics in leukocyte lineages may predict the development of complications such as graft failure/rejection, graft versus host disease (GVHD) or disease relapse after allogeneic stem cell transplantation (allo-SCT). Activated leukocytes play an important role as effector cells in the immune response. OBJECTIVE: To analyze the dynamics of chimerism in activated leukocytes (AL, CD25+) as well as its implication in the development of various complications after HLA-identical related allo-SCT.

[P740]

	Patient	Disease	Status Pre SCT	Day of CC achievement WB	T-cells	AL	aGVHD>1	ecGVHD	Relapse	Last follow up
Group 1	1	AML	2CR (No molR)	30	30	30	No	Yes	Yes, +140	exitus +9m
	2	ALL	1st CR (No molR)	15	30	15	Yes	No	No	exitus +110 pancreatitis
	3	ALL	1st CR	15	30	30	Yes	Yes	No	alive CR/CC 1 year
	4	ALL	1st relapse	15	15	30	No	DLI +50	Yes, +40	exitus +15m, in relapse CM
	5	ALL	1st relapse	15	15	30	Yes	No	No	alive CR/CC +180
	6	HD	2nd PR	30	30	30	Yes	Yes	Yes, +15m	alive after relapse CC +15mr
	7	AML	1st CR	120	120	90	No	Yes	No	alive CR/CC 1 year
	8	AML	1st CR	60	>180	90	Yes	Yes	Yes, +10m	alive CR/CC 1 year, DLI
Group 2	9	AA	PR	90	60	90	No	No	No	alive CR/CC 1 year
	10	AA	Refractory	QM	45	>180	No	No	No	alive CR/CC 1 year
	11	ALL	1st CR (No molR, No citog)	15	90	120	No	No	Yes, +210	alive in relapse +210
	12	ALL	1st CR	45	45	45	No	Yes	No	exitus sepsis +15m in CR
	13	AML	1RC (No Rmol)	30	60	60	No	Yes	No	alive CR +210 CC
	14	AML	1st CR	30	45	45	No	Yes	No	alive CR/CC +15m
	15	AML	1st CR	150	150	150	No	No	No	alive CR/CC +15m
	16	MDS	1st CR (No molR)	>180	>180	>180	No	No	Yes, +240	exitus relapse +1year

Table 1. Patient characteristics, chimerism followup in whole blood (WB), T-cells (CD3+) and activated leukocytes (AL, CD25+), developement of acute and extense chronic GVHD and clinical evolution after transplantation.

Materials and methods: Sixteen HLA-identical related Allo-SCT with a minimum follow up of 180 days were analysed. Chimerism analysis was performed by STR-PCR (AmpFlSTR SGM Plus; Applied Biosystems) in whole blood (WB), as well as in T-cells (CD3+) and CD25+ activated leukocytes (AL) purified using immunomagnetic means (Miltenyi Biotec). Complete chimerism (CC) was defined as <1% recipient in WB and <5% in leukocyte lineages (95% purity of enriched samples).
Results: Chimerism analysis in AL revealed 2 groups of patients (Table 1). Group 1: 8/16 patients who achieve early CC in AL (either CC before day +30 6/8, or earlier than in WB and T-cells 2/8). Group 2: 8/16 patients with late achievement of CC in AL (CC in AL after day +30 8/8, including 3/8 who achieved CC in LA later than in T-cells). Incidence of acute GVHD>grade I was significantly higher in Group 1 than in Group 2 (62.5% (5/8) vs 0% (0/8), p=0.017). Incidence of extense chronic GVHD showed a trend to be higher in Group 1 (75% (6/8) vs 37.5% (3/8), p=0.131). Analysis of chimerism in AL is useful for the anticipation of aGVHD>grade I since patients with MC in T-cells but with CC in AL do develop GVHD (2/8) while those who achieve CC in T-cells but maintain MC in AL do not (3/8). No relationship between the dynamics of chimerism in AL and the development of other complications was observed.
Conclusions: Chimerism dynamics in CD25+ AL affects donor/recipient alloreactivity and plays a role in the development of complications such as GVHD after HLA-identical related Allo-SCT. Analysis of chimerism in AL adds value to the analysis of T-cells for the anticipation GVHD development. Analysis of a greater number of patients will eventually allow confirming these results and establishing the usefulness of chimerism monitoring in AL for an improved management of transplanted patients.

P741
Prophylactic donor lymphocyte infusion after allogeneic haematopoieitc transplantation in patients with acute leukaemia
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Donor Lymphocyte Infusions (DLI) after allo-HCT may induce a Graft versus Leukemia (GvL) effect but with the risk of GvHD. The efficacy of DLI in acute leukemia patients exhibiting an overt relapse is limited. The value and safety of DLI administrated prophylactically after allo-HCT is not established. We report our single centre experience on 10 consecutive pts who received prophylactic DLI (pDLI) due to mixed chimerism (MC) (5pts) or because of high risk of relapse (4 ALL, 1 AML). Pts were eligible for pDLI only if did not have a history of acute GVHD≥grade II and were out of immunosuppression for >3 weeks. DLIs from sibling donors (n=5) were collected without growth factor. In order to ensure availability of DLIs from unrelated donors (VUD, n=5) a sample from the G-CSF mobilized PBSC graft was used to prepare and freeze portions of DLIs. The first pDLI was given at a median of 160 days (78-426) after HCT. The median number of CD3+ cells infused was 2,25x10^6/kg (0,7-22x10^6/kg) and the median number of infusions was 3 (1-6). All 5 patients who received pDLI because of MC turned into complete chimerism after a median number of CD3+ 1,4x10^6/kg (0,7-2,5x10^6/kg) and a median number of 2 (1-3) infusions. 60% recipients (4 sibling, 2 VUD) presented GvHD, after a median cumulative CD3+ dose of 2,25 x 10^6/kg (0,72-7 x 10^6/kg) and a median of 32 days (17-120) after the last DLI infusion. In 3 cases the GvHD was moderate and resolved (1pt acute like skin GvHD, 1pt chronic skin GvHD, 1pt gut GvHD). Further 3 recipients experienced severe post-DLI GvHD (one acute liver GvHD stage III, one liver/gut GvHD stage III and one bronchiolitis obliterans). In these clinical severe cases, GvHD occured after a median cumulative CD3+ dose of 2x10^6/kg (0.96-2.5x10^6/kg) and a median of 43 days (33-183) and 18 (11-36) days after the first and last administration of DLI, respectively. None of the 10 patients who received pDLI relapsed after a median follow up of 665 days (310-1404). 40% have died, 3/4 due to GvHD and 1/4 due to fungal pneumonia. In conclusion, we report a high incidence of GvHD after pDLI which is higher than in studies reported DLI for relapsed or progressive disease. The occurrence of severe GvHD, even after low CD3+ dose, emphasizes the need of innovative cellular strategies that enhance the GvL effect without GvHD. Prospective randomized trials evaluating the value of prophylactic DLI are warranted.

P742
Phenotypic and functional characterization of CMV-specific T CD8+ cells in HLA-A0201 patients after haematopoietic stem cell transplantation
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Introduction: Adoptive transfer of CMV-specific cytotoxic T lymphocytes (CMV-CTL) is being evaluated as treatment of refractary CMV infection after allogeneic stem cell transplantation (allo-SCT). However, CMV-CTL function relies on differentiation status: (Naïve, effector, effector-memory and central memory according to CD45RA and CCR7 expression) and also on the presence of inhibitor receptors such as LIR (Leukocyte inhibitory receptor) or PD1 (Programmed Death-1). In this study we aimed to characterize the phenotype and functional profile of CMV-CTL post-allo-SCT.
Patients and methods: We have included 26 HLA-A*0201 patients who underwent allo-SCT. Stem cell source was: bone marrow (n=12) or peripheral blood (n=14). 50% of patients received reduced intensity conditioning regimen. Peripheral blood samples were drawn after allo-SCT at time of lymphocyte recovery (>1000/µl). CMV-CTL were quantified using APC-conjugated HLA anti-pp65 tetramer and PerCP-labeled-MnAb against CD8. Phenotypic characterization was assessed

measuring the expression of CD45RA, CCR7, CD28 and CD27. Functional analyses were measured by perforin expression and inhibitory receptors LIR and PD1.
Results: 15 patients developed CMV viral replication (median DNAnemia: 26.600copies/ml) within a median of 48 days (range: 26-182) after allo-SCT. All patients harbored CMV-CTL with a median of 0.39% (range: 0.07-10.6) of total CD8+ lymphocytes and absolute number of 3.5 cells/μL (range: 0.2-183).
Patients who did not develop CMV viral replication harbored CMV-CTL with 13.4±4.8% of naïve phenotype (CD45RA+CCR7+), whereas in CMV-replication patients, naïve population account only for 2.6±1.1% (P=0.03). Likewise, percentage of naïve CMV-CTL were significantly higher if donor was ≤40 years old (P=0.02)
Regarding other differentiation subsets we found: Effector (64.7%), effector memory (27.7%) or central memory (0.35%). Perforin was expressed in 34.4% of CMV-CTL. CMV-CTL expressing inhibitor receptors CD85j or PD1 were 21.3% and 28% respectively. We did not find statistical differences comparing CMV replication or donor-age in none of maturation subsets, or in the expression of perforin or inhibitory receptors.
Conclusions: Peripheral blood anti-CMV specific CTL after allo-SCT constitute a heterogeneous population. The percentage of naive subset percentage is significantly higher when donor is ≤40 years old and when receptor has not developed viral replication.

P743

Quantitative real-time PCR evaluation of Wilms tumour gene transcript levels in autologous peripheral blood stem cell can predict the risk of acute myeloid leukaemia relapse after autologous transplantation

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Introduction: ASCT is a potentially curative option for pts with AML; unfortunately, the relapse rate after ASCT is high and can be due to contamination with leukemic blasts of autologous PBSC collected by leukapheresis (LK), although these procedures are usually performed when pts are in proved complete remission (CR). Thus, identification and quantification of a reliable minimal residual disease marker in collected PBSC could be relevant in determining the relapse risk after ASCT. High levels of WT1 transcripts detected with RQ-PCR in bone marrow and peripheral blood of AML pts in CR predict disease relapse. We retrospectively evaluated the WT1 transcript levels in autologous PBSC of AML pts who received an ASCT in CR to establish the power of this parameter to predict the risk of relapse.
Aim: to correlate the quantitative levels of WT1 in autologous PBSC with the relapse incidence in AML pts who received an ASCT in CR, at our Institute.
Patients and methods: 13 pts, all in morphological and genetic CR at the time of PBSC collection and before ASCT. PBSC collection by LK (COBE Spectra cell separator): median CD34+x10e6/kg: 9.32 (3.79-32). RQ-PCR quantification of WT1 was performed in samples of each LK, using TaqMan technology on RNA from mononucleated cells. The control gene was the housekeeping gene ABL, with WT1 level being normalised to 104 copies of ABL per sample. Conditioning regimen: treosulfan 42 gr/sqm, fludarabine 150 mg/sqm, cytarabine 10 gr/sqm. Transplant, median CD34+x10e6/kg: 5.1 (3.3-8.5).
Results: at last follow-up 6 (46%) pts have relapsed after ASCT. Median WT1 copies in LK of pts who relapsed or did not relapse were 193,87 (23.47–839.63) and 16.96 (0.59–82.49), respectively. Overall median relapse free survival (RFS) from ASCT was 455 (69-1576) days; median RFS of pts with WT1 copies >90 (n=3) or ≤90 (n=10) was 351 (93-368) and 560 (69-1576) days (log-rank p=0.003), respectively. One patient with a LK WT1 value ≤90 had an extramedullary relapse.
Conclusions: these results suggest the possibility to determine a quantitative cut-off level of WT1 transcripts in autologous PBSC, with higher levels being predictive of contamination of LK products with leukemic blasts and predicting an increased relapse risk after ASCT. These data, if confirmed by our ongoing study, will permit us to discriminate pts who can benefit from ASCT from pts who should be addressed to different therapeutic strategies.

P744

Haemophagocytic syndrome after allogeneic haematopoietic cell transplantation: more a graft-rejection than an infectious process?

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Less than 50 cases of HPS have been so far reported after allogeneic HCT, mostly within 2 months after transplant, and related to infection. We run a retrospective study within 15 EBMT centers to collect HPS cases after allogeneic HCT, according to Henter et al criteria, 1991, including > 3% hemophagocyting macrophages (Mac) in the marrow. The patients were M/F: 4/1, aged 23-65 y; had acute leukemia or myelodysplasia in 4, non-hodgkin lymphoma in 1; 4 were in complete remission; 3 received a myeloablative HCT and 2, a non myeloablative HCT, from an HLA-id (n=3), unrelated (n=1) or a cord blood (n=1) donor. HPS occurred between d26-d227. Infection was documented in 2 patients: EBV+toxoplasmosis in 1, EBV+VZV+CMV in 1. Four patients died from HPS despite treatment, including targeted anti-infectives.
One of the 3 patients without obvious infection deserves consideration. This 26-y man, transplanted from his HLA id sister for ALL in 1st remission after Cy-TBI, had an initial uneventful course, except for CMV infection at month 2 and zoster at month 3. Eight months after HCT, he developed a non-documented pneumonia which resolved with cefotaxime. He developed severe HPS three weeks later. Investigations for CMV, HSV, EBV, HHV8, H1N1, RSV, adenovirus, parvo B19, tuberculosis, leishmania, fungi, toxo, 2 BALs, were repeatedly negative. He received steroids, IVIG, VP16, rituximab, romiplostim and infliximab. All failed. A marrow aspiration at 18 months, showed the persistence of hemophagocytosis. Sex chromosome-specific FISH combined with CD68 staining on marrow showed that 6% of marrow cells, and most of the activated Mac, were of recipient (R) origin. Blood chimerism was concomitantly total D. Considering that HPS was due to a chronic reaction from R Mac to D blood cells, we gave 3 consecutive D lymphocyte infusions (DLI) to reverse chimerism to full D, including a last T-reg depleted DLI (Maury et al. 2010). Two months later, transfusion needs and marrow hemophagocytosis were decreased, marrow chimerism was full D, but thrombocytopenia persisted. He suddenly died from bacterial sepsis at 27 months.
Although HPS after allogeneic HCT is classically a peri-infective reaction, mainly due to herpes viruses, some late cases may be considered as graft rejection and treated accordingly. Therefore, once infection has been eliminated, investigating the origin (D vs R) of the activated Mac may be of outmost importance for managing the patient.

P745

Lenalidomide plus donor lymphocytes infusion after allogeneic stem cell transplantation with reduced-intensity conditioning in patients with high- risk multiple myeloma

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Introduction: Myeloma relapse is the main cause of death after allogeneic stem cell transplantation (Allo-SCT). The aim of our study is to evaluate the antimyeloma effect of lenalidomide followed of donor lymphocyte infusion (DLI) as adoptive immunotherapy after transplantation.
Patients and methods: Ten patients with refractory myeloma were analyzed. The median age at transplantation was 57 years (46-64); 5 patients (50%) received lenalidomide before Allo-SCT. All patients received a RIC including Fludarabine 30 mg/m² 5 days, ATG 2,5 mg/kg for 2 days and Busilvex 3.2 mg/kg/day (3 days in 6 patients and 2 days in 4 patients). All but one received peripheral blood stem cells (PBSC). Donor was HLAid in 6 patients and matched unrelated in 4 patients.
Patients were treated by lenalidomide if myeloma was progressive or residual disease was observed at day +100 and if no GVHD signs were evident. Dosage was variable between 10 and 25 mg/day. DLI were administered after at least 2 cycles of lenalidomide.
Results: The median time between Allo-SCT and lenalidomide was 6 months (3-12). The median initial dose of lenalidomide was 15 mg (10-25). The patients received a median of 5 cycles (1-9). 6 patients (60%) received an escalating dose of DLI; 1 x 10^7/Kg of CD3+cells for the first DLI and 1 x 10^8/Kg of CD3+cells for the second DLI. One patient with GVHD and one patient with progressive disease after lenalidomide did not receive DLI. Two patients are waiting for DLI.
The toxicity related to lenalidomide was haematological toxicity grade II in 4 patients (40%) and grade I in 2 patients (20%); 6 patients (60%) had moderate asthenia. None of our patient developed a renal insufficiency or thrombo-embolism.
At the last follow up, 9 patients are alive and 8 of them are on treatment. 2 patients achieved complete remission (CR) and 6 patients partial remission. The 1 year probability of overall survival (OS) was 90 % and the progression-free survival (PFS) was 80%.
Conclusions: These data show that i) lenalidomide is well tolerated after Allo-SCT; ii) the association with DLI did not offered a higher risk of GVHD; iii) an immunological synergistic effect. This combination should be further evaluated in a larger cohort of patients.

P746

T-cell chimerism does significantly bias overall chimerism status after T-cell depleted allogeneic stem cell transplantation and is influenced by immunological factors including the conditioning regimen, GvHD and CMV serostatus

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Mixed chimerism (MC) after T cell depleted (TCD) allogeneic stem cell transplantation (alloSCT) is frequently measured in bone marrow (BM) leukocytes and is thought to reflect the persistence of malignant hematopoietic cell lineages. Most patients (pts) are transplanted for myeloid or B cell malignancies and it is unclear whether MC in BM leukocytes truly reflects the persistence of recipient cells in these B or myeloid cell lineages, since the total leukocyte chimerism is a composite measurement of chimerism status in different hematopoietic cell lineages. We hypothesized that leukocyte MC may be strongly influenced by T cell chimerism. Since T cell chimerism probably reflects survival and expansion of residual recipient and/or donor T cells, we hypothesized that T cell chimerism is influenced by immunological factors controlling T cell expansion. To test these hypotheses, detailed lineage specific chimerism analysis was performed 3 months after TCD alloSCT in 60 pts with hematological malignancies. In 72% of the pts analyzed, MC was detected in the T cell compartment, with a median percentage of 19% (range 1-100). In the BM leukocyte compartment, 47% of the pts were MC with a median percentage of 3% (1-22). Of the pts with MC in BM leukocytes, 24% showed MC in the T cell compartment and complete donor chimerism in the B and myeloid compartment. A significant lower percentage of recipient T cells in myelo-ablative (MA) transplanted pts (1% (0-100) was detected, as compared to non MA transplanted pts (32% (0-100). In MA pts transplanted with a non related donor (receiving additional alemtuzumab (ALT) as part of the conditioning regimen) a lower percentage of recipient T cells was detected (0% (0-8) as compared to MA pts transplanted with a related donor not receiving additional ALT (17.6% (0-100). Pts developing GvHD showed a significant lower percentage of recipient T cells (1% (0-100) as compared to pts without GvHD (52% (0-100). CMV seropositive pts pre-alloSCT showed higher percentages recipient CD8 T cells (58% (0-100) as compared to CMV seronegative pts (2% (0-100), both transplanted with a CMV seronegative donor. In conclusion, these results illustrate that BM leukocyte chimerism was strongly influenced by T cell chimerism, which was in turn influenced by the conditioning regimen, GvHD and CMV serostatus. Therefore, the persistence of recipient malignant hematopoietic cell lineages should be analyzed using lineage specific chimerism analysis.

P747

Plasmacytoid dendritic cell reconstitution is faster after cord blood than after bone marrow transplantation

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Natural killer (NK) and plasmacytoid dendritic cells (pDC) are innate immune cells that orchestrate the immune defense against virus and tumoral cells. After allogeneic stem cells transplantation, both pDC and NK cells have been shown to independently influence transplant outcomes. However, none study have directly evaluated the reconstitution of pDC and NK cells depending on the stem cell source. We thus aim to investigate the reconstitution and the functionality of the pDC/NK axis after umbilical cord blood (UCBT) versus bone marrow transplantation (BMT) in children treated for malignant or non-malignant diseases. Absolute numbers and phenotypes of pDC and NK cells were monitored by flow cytometry during the first year post transplantation. pDC and NK cell activations were investigated on whole blood cells following overnight in vitro stimulation with CpG oligonucleotides or IL-15 and IFN-α respectively. We show that the kinetic of NK cell reconstitution is similar after UCBT and BMT and is characterized by a rapid increase of blood NK cell numbers associated with a predominance of CD56bright subset. After in vitro stimulation, NK cell activation was not different in patients treated with UCBT or with BMT. Interestingly, blood pDC counts reached higher levels following UCBT than following BMT as early as 2 months post transplantation and these counts remained elevated during the first year post-transplant. We also revealed a higher production of IFN-α by unstimulated blood cells from patients treated with UCBT as compared to patients treated with BMT. This constitutive IFN-α production could not be increased by in vitro stimulation with CpG oligonucleotides. In our cohort, no significant association between pDC recovery and patient outcome following UCBT could be identified. Collectively, these data show that the pDC/NK axis is functional following UCBT and therefore could represent an appropriate target for innate immunity-based immunotherapy.

P748

Lymphocyte recovery and infused CD34+ cells dose: effect on the evolution after autotransplantation

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Objectives: The number of infused CD34+ cells (CD34+i) has been associated with early absolute lymphocyte count (ALC) and the outcome undergoing autologous haematopoietic stem cell transplantation (HSCT) in patients with hematologic malignancies. ALC < or = 500/µl on day 15 post-HSCT has been proposed as an independent risk factor in the evolution of these patients. The aim of our study is to analyze the relationship between the number of CD34+i and the days hended to reach ALC > or = 500/µl and assess its influence on achieving a ALC>500/µl on day 15 and the evolution.
Methods: Medical records of 163 patients receiving autologous-HSCT between January 2003 and March 2010 in La Paz University Hospital were reviewed. The analysis of the relationship between CD43+i cells and time to ALC > or = 500/µl was performed by the Spearman correlation coefficient (r) and ANOVA test, we also applied Cox regression model, ROC curves and survival curves of Kaplan-Meier.
Results: Median CD34+i cells was $2.99x10^6$/Kg(range:0.54-26.23) and the median of days required to ALC > or = 500/µl was 14 (range:3-30). We found significant and inversely proporcional relationship between the number of CD34+i and the days hended to reach ALC > or = 500/µl in patients with NHL ($r = -0.625$; $p < 0.001$), HL ($r = -0.801$; $p < 0.001$) and MM ($r = -0.662$; $p < 0.001$) according to regresion line: days = -0.981 x number of CD34+ i + 18.09. Infusion of CD34+ cells > or = $2.0x10^6$/Kg relates directly and significantly to ALC>500/µl on day 15 (RR = 7.77; $p < 0.001$ (IC95%:5.55-90.25)) and post-HSCT survival so that ALC > 500/µl (n=97(59.5%)) is associated with better progression-free survival (PFS) and overall survival (OS), than those patients with ALC below this limit (n = 66 (40.5%)): PFS 67 months (CI95%:62-73) vs.23 months (CI95%:15-31) p<0.001 and OS 82 months (CI95%:78-85) vs.55 months (CI95%:44-65) p < 0.001. Multivariate analysis showed that not reaching an ALC > 500/µl on day15 is an adverse factor for PFS with HR 7.7 (CI95%:4-14.8) p<0.001, and for OS with HR 9.8 (CI95%:3-30.3) p<0.001.
Conclusions:

1) The study shows correlation between CD34+i and cellularity and kinetics of lymphocyte recovery, more evident in patients with LH
2) We confirm the positive prognosis impact of early ALC>500/µl on the evolution after autotransplantation
3) Patients with less than $2.0x10^6$/Kg CD34+i, have 8 time less probability to achieve an ALC>or=500/µl on day15 and therefore a worse prognosis
4) We have obtained a predictive model of lymphocyte recovery based recovery of CD34+i.

P749

Patterns of phagocytic activity of monocytes, granulocytes and lymphocytes after haematopoietic stem cell transplantation

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Background: Transplant related adverse events are a major cause of morbidity and mortality in pediatric patients after high dose chemotherapy and hematopoietic stem cell transplantation (HSCT). T cell depletion of the graft, post transplant immune deficiency, extensive immunosuppression, and profound granulocytopenia are known risk factors for life threatening bacterial, viral, fungal infections as well as acute graft versus host disease (GvHD). Phagocytosis presents a major mechanism contributing to the clearance of pathogens and cell debris. We hypothesized that phagocytic activity might likewise be altered during adverse events after HSCT. We conducted a pilot study and analyzed the phagocytic activity of monocytes, granulocytes and lymphocytes before, during and up to one year after HSCT and in particular during transplant-related adverse events.
Patients and methods: The patient group consisted of 25 immune compromised pediatric patients and young adults with hemato-oncological malignancies and immunodeficiency disorders (median of age 9.0 years). Normal values of phagocytic activity of granulocytes, monocytes and lymphocytes were analyzed from a control group of healthy children and young adults (n = 36). The period of analysis began with the day before the conditioning regimen was started until 365 days after HSCT. Phagocytic activity was determined by FITC marked Escherichia coli bacteria by flowcytometric analysis.
Results: The median analysis period in the patient group was 213 days (range 45-382 days). After HSCT the phagocytic activity of monocytes, granulocytes, and lymphocytes was not altered in general. However, during the conditioning period, a significant decrease of phagocytic activity of monocytes was observed along with a significant increase of phagocytic activity of granulocytes. During adverse events after HSCT, we observed a diverse pattern of phagocytic activity. A significant increase occurred during sepsis and bacterial infection, however not during viral or fungal infection. The occurrence of GvHD also did not lead to changes in phagocytic activity of monocytes, granulocytes and lymphocytes.
Conclusion: During adverse events after HSCT diverse patterns of phagocytic activity of monocytes and granulocytes could be observed. We conclude that these alterations contributes to an altered immune response during adverse events after HSCT.

P750

Successful engraftment after allogeneic haematopoietic cell transplantation in patients with myelofibrosis after treatment with the JAK2-inhibitor INCB018424

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Clinical symptoms of myelofibrosis (MF) include progressive splenomegaly, cytopenias and constitutional symptoms. MF could also progress to blast transformation and finally to the death of the patient.
Management of patients with MF is a challenge to hematologists. Treatment options are limited. Allogeneic hematopoietic cell transplantation (HCT) remains the only curative treatment approach. However, such therapy is complicated by a high non-relapse related morbidity and mortality. The recent discovery of the JAK2V617F mutation in patients with myeloproliferative neoplasias was followed by the development of several JAK2-inhibitors that are currently being evaluated in clinical studies.
We report on 3 patients (pts) with MF (Pt 1 (48 years (y), female)=JAK2V617F negative, Lille score 2; Pt 2 (57 y, female)=JAK2V617F negative, Lille score 2; Pt 3 (66 y, male)= JAK2V617F positive, Lille score 1). Therapy with the JAK2-inhibitor INCB018424 (15 mg BID) was started in December 2009. After a median of 7 (range 5-10) months of JAK2-inhibitor-therapy, an allogeneic HCT from a mismatched unrelated donor in Pt 1 and Pt 3 and a HLA identical sibling in Pt 2 has been performed because of persistent splenomegaly in two and progressive leucocytosis in the third patient. Conditioning regimens comprised cyclophosphamide (Cyclo, 60 mg/kg/d over 2 days), total body irradiation (TBI, 12 Gy over 3 days) and Thymoglobulin (2 mg/kg/d over 3 days) in Pt 1, Cyclo (60 mg/kg/d over 2 days) and Busulfan (16 mg/kg/d over 4 days) in Pt 2, and Fludarabine (30 mg/m²/d over 3 days) and TBI (2 Gy on day 0) in Pt 3. A median of 6.5 x 10^6/kgKG (range 5.47-7.9 x 10^6) peripheral hematopoietic progenitor cells mobilized from peripheral blood were infused. At a median follow up of 4 (range

2-7) months post-transplantation course was unremarkable. Hematologic engraftment was on time with ANC>0.5/nl on day 18 (range 14-21) and platelets>20/nl on day 17 (range 14-22). Marrow examination on day +28 for chimerism analyses monitored by PCR of polymorphic micro satellite regions showed a median of 90% (range 77-100) donor cells.
We show that allogeneic HCT after treatment with the JAK2-inhibitor INCB018424 is feasible and does not preclude successful engraftment. Prospective studies are required to determine whether or not the Jak2-inhibitor is able to reduce pre-transplant morbidity and/or non-relapse related morbidity and mortality after HCT.

P751

Resistant pure red cell aplasia after allogeneic stem cell

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ABO-mismatch is not a contraindication for haematopoietic stem cell transplantation (HSCT) but it is associated with specific problems. Delayed donor red cell engraftment and pure red cell aplasia (PRCA) are rare, but well-known complication of major ABO-incompatible HSCT. It is associated with inhibition of donor erythroid progenitors by residual host isohemagglutinins.
We report a 50-yeas-old woman with acute myeloid leukemia who developed PRCA after HSCT. Her sibling donor was HLA identical and ABO incompatible. Patient had blood group O(I)Rh+ before transplantation, bone marrow donor was A(II)Rh+. Conditioning regimen was non-myeloablative (fludarabine 180 mg/kg + busulphan 8 mg/kg + ATG 40 mg/kg). The white blood cells recovered more than $1.0x10^9$/l at day +20 after HSCT, platelet cells number was more than $50x10^9$/l at day +17 after HSCT. There was hematological and molecular remission during all observation time after HSCT. But there was no red blood cells precursors in the bone marrow 1 and 2 months after HSCT. There was no more than 0-1‰ reticulocytes in blood and there was no red blood cells of donor's type A(II). Patient had severe anemia and she was transfusion-dependent. Isohemagglutinins α and β; were detected during observation time. 2,5-3 months after HSCT five procedures of plasmapheresis were done without any response. 112 days after HSCT rituximab was administrated in the dose 200 mg/m² (300 mg). All therapy was ineffective.
It was decided to adsorb α-isohemagglutinins with adsorption columns – ABO Adsopak®-A (Pocard, Russia). Seven procedures of immunoadsorption were done (152, 154, 159, 171, 174, 187 and 199 days after HSCT). No α-isohemagglutinins were detected after the third procedure. The reticulocytes number increased to 40‰. 178 days after HSCT red cells A(II) appeared (5%). Red blood cells precursors in the bone marrow became 20%. The day +206 after HSCT donor's type red cells constituted to 90%. No complications were registered. So we noted fast red cells recovery.
Immunoadsorption was very effective and it shoud be considered a treatment option for resistant PRCA.

P752

Analysis of lymphocyte subset reconstitution in patients with allogeneic haematopoietic stem cell transplantation and influence of pre-transplant CMV serological status. A single-centre experience

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Introduction: Delayed immunologic reconstitution in the recipient after allogeneic haematopoietic stem cell transplantation (HSCT) represents a major concern for the long-term follow up due to its role in post-transplant infectious morbidity, including viral reactivations.
Objectives: This single center retrospective study addresses the issue of immunologic reconstitution of lymphocyte subsets after allogeneic HSCT and its potential relationship to the pre-transplant cytomegalovirus (CMV) serological status.
Patients and methods. 16 patients receiving allogeneic HSCT in our center between 2003 and 2009 have been enrolled in this study. Three patients with severe aplastic anemia, 6 with acute myelogenous leukemia, 5 with acute lymphoblastic leukemia, 1 with myelodysplastic syndrome and 1 chronic granulomatous disease. The immune reconstitution was assessed for B, T and NK cells by flowcytometry at 1, 3, 6, 9, 12 months and at 2 years post-transplant.
Results: 5 –year overall survival of the cohort was 87.5%. The most delayed reconstitution characterized CD3+ CD4+ lymphocytes with a median time to reach the 10th percentile absolute value for age (p10 AV) at 9.2 months, although earlier in younger patients. The NK cell reconstitution was delayed as well, with a median time to reach p10 AV at 5.1 months. Regarding the CD19+ compartment, they reached p10 AV at a median time of 4 months. A percent of 12.5 % of the recipients were CMV negative whereas 18.75% of donors were CMV negative. One single donor-recipient pair was CMV negative. Patients receiving grafts from CMV positive donors had interestingly earlier and enhanced recovery of CD8+ T cells, as compared to patients transplanted from CMV negative donors.
Conclusions: Reconstitution of CD8+ lymphocytes was enhanced in recipients receiving grafts from CMV positive donors as compared to grafts from CMV negative donors, whereas CD4+ cells had a rather delayed reconstitution in the whole cohort, especially in elder patients.

P753

Does level of chimerism predict graft rejection and graft failure in HSCT? – A single-centre experience

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Post-transplantation events such as GVHD, graft rejection (GR) and disease relapse are major complications in Hematopoietic Stem Cell Transplantation (HSCT). Thus their early prediction is of a significant importance for patient's surveillance. Chimerism analysis is considered as a powerful tool which allows close monitoring of the engraftment and contributes to better outcome of the transplantation. Here, we evaluate the predictive role of chimerism as well as the relation between the disease and the chimerism status.
Materials and methods: We analyzed 39 patients (12 from MUD, and 27 from MRD) with blood malignant and non-malignant diseases that were transplanted in the period 2005-2010 - 16 patients with ALL, 12 with AML, 1 with CML, 7 with Thalassaemia and 3 with ASAA. 34 recipients were transplanted from HLA matched donor and 5 from mismatched donor. Stem cell sources were bone marrow (4), peripheral blood stem cells (33), and cord blood (2 patients, 3 units). All patients and donors were previously typed for HLA-A, -B, -C, -DRB1, and –DQB1 using PCR-SBT technique. Chimerism levels were defined by PCR-STR method.
Results: Complete donor chimerism (donor cells >99%) was achieved in 62% of patients (83% of patients with malignant diseases and 17% of non-malignant cases), whereas persistent mixed chimerism was more common in non-malignant patients – 30% vs. 17%. Additionally, in 4 of the patients with non-malignant diseases (40%) a graft rejection was observed compared to only 2 of the cancer patients (7%). In 4 of those cases, GR could be predicted much earlier than it was diagnosed by the physicians. Prediction was based on the chimerism results

showing progressive increasing of MC or its complete absence. The fifth patient had developed GR before a chimerism sample was sent to the laboratory. Fifteen patients developed GVHD – 14 with malignant diseases (8 ALL and 6 AML), and only one with non-malignant (Thalassaemia). All of them were transplanted from PBSC. Cord blood units had been transplanted to 1 patient with AML (2 CB units from MUDs) and 1 with Thalassaemia (1 CB unit from MRD). Both of them developed graft rejection approximately 100 days after transplantation.
Conclusion: Our study showed the importance of chimerism analysis for the outcome of HSCT, especially to predict graft rejection and failure. A relation between the MC and malignant and non-malignant diseases could be speculated.

Infectious complications

P754
Antagonism of vasoactive intestinal peptide activity stimulates anti-viral immunity and protects transplant recipients from murine cytomegalovirus infection
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Background: Vasoactive intestinal peptide (VIP) induces regulatory dendritic cells (DC) that limit graft-versus-host disease (GvHD) in allogeneic hematopoietic stem cell transplantation (allo-HSCT) recipients. We recently reported that VIP knockout (KO) mice and transplant recipients having VIP KO hematopoietic cells are resistant to murine murine cytomegaolovirus (mCMV) infection and have improved kinetics of anti-viral immune responses. We hypothesized that selective targeting of VIP signaling in immune cells could enhance anti-viral and anti-tumor immunity in murine models of mCMV infection.
Methods: Anti-viral immunity in syngeneic HSCT recipients transplanted with VIP-KO BM and graded doses of VIP-KO T-cells was determined by infecting them with lethal doses of mCMV. Anti-viral immunity in normal C57BL/6 (B6), B6 VIP KO and/or Balb/c mice were treated with 10µg VIP antagonist/mouse or PBS s.c. daily for 8 days and challenged with lethal doses (LD50 and LD90) of mCMV on 2nd day of treatment. Viral load/liver on day 3, 10 and 18 post infection was measured by a plaque assay; anti-viral T-cells were measured by FACS using mCMV-peptide-specific MHC tetramer.
Results: MCMV infected VIP-KO mice and syngeneic recipients of VIP-KO hematopoietic cells had faster viral clearance compared to WT mice and syngeneic recipients with WT hematopoietic cells. Increased viral clearance is associated with increased numbers and enhanced cytolytic activity of IFN-g+ NK cells and more Th1/Tc1 polarized and CMV-tetramer+ CD8+ T-cells, with fewer IL-10+ and PDL1+ T-cells compared with WT T-cells. Allogeneic HSCT recipients with VIP-KO BM and graded doses of VIP-KO T-cells also showed enhanced anti-viral immunity without increasing GvHD. Subcutaneous administration of VIP antagonist for eight days in normal B6 or Balb/c mice had better survival following lethal doses of mCMV infection (Figure 1), had >90 percentage reduction of viral load/liver and had increased numbers of IFN-g+ NK and NKT cells per spleen compared to the mice treated with PBS. Daily injections of VIP antagonist did not cause any deleterious effect compared with the PBS-treated mice.
Conclusion: VIP signaling inhibits regulatory immune mechanisms and increases innate and adaptive anti-viral immunity without increasing GvHD. Selective targeting of VIP-signaling represents a novel therapeutic approach to enhance anti-viral immunity in the settings of immunodeficiency and allo HSCT.

P755
Cidofovir is effective and well tolerated in the treatment of patients with ganciclovir refractory cytomegalovirus infection after allogeneic stem cell transplantation
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Introduction: Cytomegalovirus (CMV) infection and disease continue to represent significant complications after allogeneic stem cell transplantation (SCT). Ganciclovir (GCV), whether

[P754]

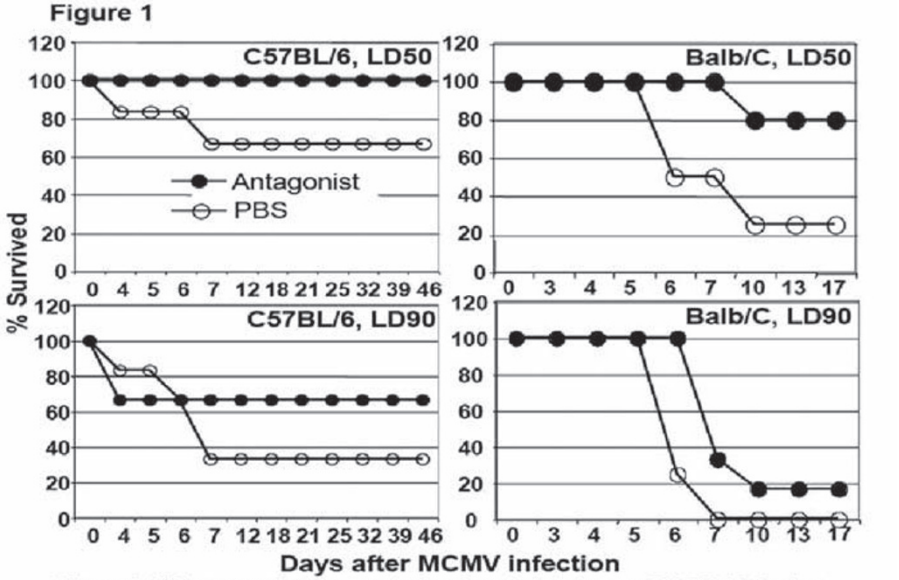

Figure 1. VIP antagonist protected mice from lethal doses of MCMV infection. C57BL/6 and Balb/C mice (n=6) were treated with 10 µg VIP antagonist s.c for eight days. They were challenged with lethal doses (LD50 and LD90) of MCMV on 2nd day of VIP antagonist administration I.p. % Survival of post infected mice are shown.

administered pre-emptively or prophylactically, is associated with substantial haematological toxicity and new treatment strategies are required. Cidofovir (CDF), a nucleotide analogue with a broad anti-viral spectrum, has proven activity in patients with CMV disease but there is limited data concerning its role in the management of CMV infection refractory to GCV. We have therefore studied the tolerability and clinical activity of CDF in 55 patients with GCV refractory CMV infection occurring after allogeneic SCT.
Patients and methods: 55 patients were treated with CDF at a dose of 5mg/kg weekly adjusted for renal function. The median number of doses of CDF administered was 4 (range 1-19) and the median starting dose was 277.5mg (3.8mg/kg). Indications for commencement of CDF therapy were persistent viraemia after 2 weeks of GCV therapy (n=35), cytopenia associated with GCV treatment (n=11), CMV disease developing on GCV therapy (n=7) and CMV reactivation prior to neutrophil engraftment (n=2). 50 patients had received in vivo alemtuzumab (10 mg/day x 5 days) in addition to ciclosporin as GVHD prophylaxis. In 29 patients donor and recipient were CMV seropositive and in 26 the patient but not the donor was CMV seropositive.
Results: 43 (78%) patients treated with CDF cleared CMV viremia and discontinued all anti-viral therapy. Of note 7 of 8 patients with CMV disease responded with disease eradication. 8 (15%) patients died before clearance of CMV infection but only one patient died of CMV disease. The major complication of CDF therapy was renal toxicity: in 13 patients (24%) the serum creatinine doubled and 3 patients required renal dialysis. The incidence of Grade 3 or 4 neutropenia was 9% and no patient developed graft failure requiring stem cell reinfusion. 27/55 patients treated with CDF are alive with a median follow up of 19 months (range 3-62 months).
Conclusion: CDF is an effective therapy in patients with GCV refractory CMV infection and has notable activity in CMV disease. Renal toxicity in this complex patient population appears to be acceptable and there is only modest haematological toxicity. CDF should be considered standard of care in patients with GCV refractory CMV infection after allogeneic transplantation.

P756
Post-transplant CMV reactivation after CBT: the role of delayed immune reconstitution or donor's seronegativity?
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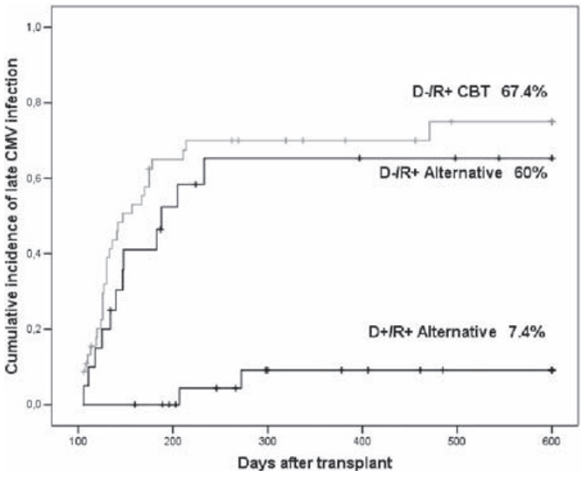
Allogeneic cord blood transplantation (CBT) is being increasingly used but immune recovery is reported as delayed. The aim of this study is to compare CMV infections in CBT recipients with transplants from unrelated or family mismatched donors (referred to as alternative donors).
CMV infection was monitored by pp65 antigenemia, late infection was defined as > 100 days after HSCT. The cumulative incidence (CI) and survival were calculated by means of Kaplan-Meier method, log rank test. Overall, 136 consecutive transplants in seropositive recipients were identified and divided according to donor type ad serostatus into:
1) 38 D+/R+ alternative transplant,
2) 29 D-/R+ alternative transplant and
3) 69 D-/R+ CBT. Median follow-up was 257 days (1-1328).
CI of CMV infection was slightly higher in D-/R+: D-/R+ alternative 69%, D-/R+ CBT 72.5% and D+/R+ alternative 55.3%, p=0.2). Late infection was significantly more frequent in D-/R+ group: D-/R+ alternative 60%, D-/R+ CBT 67.4% and D+/R+ alternative 7.4%, p<0.001, figure 1). The time from the first to last positive antigenemia was 11 days (range, 1-249) for D+/R+ alternative group versus 126 days (range, 1-794) for D-/R+ alternative group (p=0.006), and versus 106 days (range, 1-674) for D-/R+ CBT (p<0.0001). There was no difference in overall survival between D+/R+ and D-/R+ groups (p=0.98).
In conclusion, characteristics of CMV infection were similar in D-/R+ CBT and D-/R+ alternative transplant group, and differed importantly from D+/R+ alternative transplant group. The burden of CMV morbidity, related mostly to late infection, seems to be associated rather with donor serostatus than with CBT-related immunodeficiency.



P757
Immunomonitoring of adenovirus infectious risk and of specific cytotoxic lymphocytes infusion in paediatric haematopoietic stem cell transplantation
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Background: The profile of adenovirus (AdV)-specific T-cells immune recovery that could best predict the capacity of immunocompromised host to fight AdV is unclear.
Methods: To this aim, 47 paediatric patients were enrolled for at least 3 months at time of hematopoietic-stem cell transplantation (HSCT) either with genoidentical (n=23), unrelated (n=18 of which nine 10/10 and nine 9/10 HLA-matched) or unrelated cord-blood transplantation (n=6). Enumeration of AdV-specific CD4 T-cells secreting cytokines (flow-cytometry) and proliferative-responses to AdV (3HT-incorporation) were evaluated at least at month 1, 3 and 6 post-HSCT and linked to AdV-DNAemia (performed weekly the first 3 months) and/or to clinical symptoms.
Results: i) 44/47 patients did not evidence AdV-DNAemia. 32 out of 44 (73%) developed CD4-mediated IFNg-responses to AdV (median 0.36 CD4/µl of blood) since the first month post-HSCT (n=11: 8 genoidentical and 3 unrelated) or the third month (n=21 additional patients). At 3 months, both incidence and level intensities of AdV-specific CD4 appeared similar in genoidentical and unrelated BMT (70% and 80%; 0.36 and 0.21 CD4/µl, respectively) and not statistically different from age-matched controls (76%; 1.35 CD4/µl) whereas cord-blood transplanted patients exhibited similar incidence but higher level intensities (67%; 1.49 CD4/µl). Polyfunctional (IL2+/IFNg+) and proliferative responses appeared later since the third month.
ii) Three out of four 9/10 HLA-matched unrelated HSCT that did not develop immunity to AdV presented chemotherapy resistant AdV-DNAemia at 3 to 5 months post-HSCT and subsequent AdV-related severe diseases. Two were treated with AdV-specific cytotoxic lymphocytes (CTL) infusion. AdV-PCR became negative while IFNg responses to the virus developed, followed by the development of long-lasting proliferative and polyfunctional IL2+/IFNg+ responses.
Conclusion: Monitoring, since month 1 post-HSCT, of IFNg-secreting AdV-specific CD4 appears suitable for early detection of patients at low risk. Undetectable responses at month 3,

in 9/10 HLA-matched unrelated HSCT, could confer a risk of severe infection. Polyfunctional cytokine responses could be used as a signature of long-term protection.

P758

Respective performance of a proliferative and a cytokine assay to monitor influenza vaccine immunogenicity

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Objective: In pediatric hematopoietic-stem cell transplantation (HSCT), analysis of vaccine immunogenicity could use cellular instead of humoral assays to avoid IgIV interference. No standardized and reproducible cellular assay is available to do so. Here, we evaluated comparatively the performance of a proliferative and a cytokine production assay for the monitoring of responses to influenza in children vaccinated within the first year post-HSCT.
Methods: 28 HSCT recipients, aged 4.8y (1.4-16.9) were transplanted after myeloablative conditioning, during the 2009-2010 influenza season. 14 were vaccinated with one (n=3) or two (n=11) doses of the adjuvanted (Pandemrix®, n=6) or non-adjuvanted (Panenza®, n=8) pandemic H1N1 vaccine at 171 days (76-330) post-HSCT; 4 were also vaccinated with the seasonal vaccine (Vaxigrip®). 14 were not vaccinated, of whom one developed H1N1 flu. 7 healthy H1N1-vaccinated individuals >13y of age were used as controls. T-cell proliferation (3HT incorporation) and enumeration, by flow cytometry, of IFN-g and IL2 secreting CD4+ T-cells in response to inactivated H1N1 and H3N2 viruses were compared in the four groups.
Results: i) 12/14 (86%) vaccinated HSCT evidenced proliferative responses to H1N1 with stimulation index (SI) ≥ 9 of whom 11 also responded to H3N2, irrespective of vaccination with Vaxigrip® (n=4) or with the only H1N1 vaccine (n=7). Incidences and intensity levels of proliferative responses to H1N1 and to H3N2 were similar in vaccinated HSCT and controls.
ii) In contrast, only 2/13 (15%) non-vaccinated and non-infected HSCT recipients had SI≥9 to H1N1 and /or H3N2 and responses intensity levels were lower ($p<0.003$) compared to vaccinated HSCT.
iii) Cytokine responses to H1N1 and to H3N2 reached the threshold of detection respectively in 67% and 56% of vaccinated HSCT but in 29% and 100% of vaccinated controls. When observed, cytokine responses involved predominant IL2+/IFNg+ responses in controls but IL2+/IFNg- in vaccinated HSCT.
iv) Finally, high proliferative and cytokine responses to both H1N1 and H3N2 were observed in the only flu-infected HSCT recipient in whom IL2+/IFNg+ responses predominated.
Conclusion: Proliferative assays could be more sensitive than cytokine assays to monitor inactivated influenza vaccine. Both assays revealed heterosubtypic responses to H1N1 vaccine. Further studies are required to link together distinct profiles of cytokine responses and protection.

P759

Clinical features and outcome of 2009-influenza A (H1N1) after allogeneic haematopoietic stem cell transplantation

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Objectives: The impact of the 2009 H1N1-Influenza A (H1N1) pandemic in allogeneic hematopoietic stem cell transplant recipients (allo-HSCT) is not yet well defined. The aim of this study was to better characterize the features and outcome of this novel infection among adult allo-HSCT recipients.
Methods: Between May 2009 and May 2010, all allo-HSCT who presented with respiratory symptoms were PCR-screened for the presence of H1N1 virus. Oseltamivir resistance and viral loads were assessed at the discretion of the treating physician. Complete clinical and biological chart reviews were performed for all cases.
Results: During the study period, 10 of 248 patients followed at the Geneva University Hospital had a confirmed H1N1-infection, representing an overall incidence of 4% (95% CI, 2-7%). Close contact with children was the most commonly suspected mode of transmission (50%). With regards to their immunological status, all patients but one had been vaccinated against seasonal influenza and 5 against H1N1-influenza. Upper and lower respiratory tract infection were present in 8 and 5 patients, respectively. The median duration of symptoms before diagnosis confirmation was 2.5 (range 0-15) days. Lymphopenia < 1G/l was the most frequent biological abnormality (60%). All patients received oseltamivir that was initiated at a median of 3 (range, 0-8) days after symptoms onset for a median duration of 6.5 (range, 5-20) days. Six patients had other associated viral or bacterial pathogens in respiratory secretions. Eight

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Table 1: Baseline and clinical features of H1N1 infected patients at presentation

Pts	Sex/Age Malignancy	Donor type Cond Reg	Karnofsky Score/Chronic conditions	GvHD IS/Cs	Baseline CD3/CD4/CD8 (/µl)	Time between H1N1 vaccine and symptoms onset (D)	Time since transplant (M) / Symptoms duration before diagnosis (D)	Rhinorrhea/ Sore throat/ Dyspnoea/ Myalgia	URTI LRTI	Associated respiratory copathogens	LOS (D)/ Mechanical ventilation duration (D)	Oseltamivir/ zanamivir duration (D)	H275Y mutation / Viral shedding (D)	Outcome
UPN 1	F/23 HL	Sib RIC/UM	100/None	None	1423/310/1078	-	8/4	Y/N/N/Y	Y N	None	5/-	5/-	NA	Full Recovery
UPN 2	M/26 ALL	MUD MAC/T-dep	100/None	None	399/164/266	+1	6/1	Y/N/N/Y	Y N	RSV Adenovirus	-	5/-	N/NA	Full Recovery
UPN 3	F/32 CML	Sib MAC/T-dep	100/None	None	1089/744/264	+7	109/2	Y/Y/N/Y	Y N	None	-	5/-	N/NA	Full Recovery
UPN 4	M/49 MM	MUD MAC/UM	70 /None	Extensive Y/30	37/11/22	-	12/4	Y/N/Y/Y	Y Y	Klebsiella Oxytoca	50/43	15/15	Y/12	Expired D+54
UPN 5	F/62 AML	Sib RIC/T-dep	80/Diabetes	Extensive Y/80	718/352/381	-24[1]	8/15	N/N/Y/N	N Y	None	20/6	13/8	N/19	Full Recovery
UPN 6	M/57 NHL	Sib RIC/UM	100/Esophageal cancer	None	NA	-19	86/3	Y/Y/Y/Y	Y N	Picornavirus	-	6/-	N/23	Full Recovery
UPN 7	M/40 AML	Sib MAC/T-dep	70/Diabetes, Renal, Pulmonary	Extensive Y/30	861/121/691	-	52/1	Y/N/Y/N	Y Y	CMV EBV	90/70	20/20	Y/21	Expired D+94
UPN 8	M/56 ALL Ph+	Sib RIC/UM	80/Pulmonary	Extensive Y/30	1005/376/646	-10[1]	22/4	Y/Y/Y/N	Y Y	None	-	5/-	N/NA	Full Recovery
UPN 9	M/52 CMML	Sib MAC/T-dep	100/LGL, Splenoctomised	None	6630/580/5967	-	18/2	Y/Y/Y/N	Y N	RSV	-	7/-	NA	Full Recovery
UPN 10	M/53 AML	Sib RIC/T-dep	70/Diabetes, Renal, Pulmonary	Acute Y/60	141/18/115	-	8/2	N/N/N/Y	N Y	Picornavirus	15/-	15/15	NA	Full Recovery

[1]UPN 5 and 8 had 2 vaccine doses (only time since first dose is specified)
Abbreviations: ALL : Acute Lymphoblastic Leukaemia; ALL Phi+: Acute Lymphoblastic Leukaemia Philadelphia positive; AML: Acute Myeloid Leukaemia; Cond Reg: conditioning regimen; CML: chronic Myeloid Leukaemia; CMML: Chronic Myelo-monocytic Leukaemia; CMV: cytomegalovirus; D: days; EBV: Epstein-Barr virus; F: Female; GvHD: graft-vs-host disease; HL: Hodgkin lymphoma; IS/Cs: immunosuppressive drugs/corticosteroids (prednisone equivalent mg/day); LOS: length of stay; LRTI: lower respiratory tract infection; M: male ; M : Month ; MAC: myeloablative conditioning; MM: Multiple Myeloma; MUD: Matched unrelated donor; N : No ; NA : Not Available ; NHL: Non Hodgkin Lymphoma ; Pts : patients ; RIC: reduced intensity conditioning; RSV: respiratory syncytial virus; Sib: identical sibling; T-dep: T-cell depletion; URTI: upper respiratory tract infection; UM: unmanipulated graft; VZV: varicella-zoster virus ; Y : Yes

received systemic antibiotics in addition to antiviral treatment. Three patients with significant comorbidities and graft-versus-host disease had severe infection with prolonged viral shedding and required mechanical ventilation. In those patients, IV zanamivir was given in addition to oseltamivir for a median period of 15 (range, 8-20) days. Sequencing of the neuraminidase gene showed an oseltamivir resistant strain in 2 patients who died subsequently despite intensive therapy, giving a case fatality rate of 20%.
Conclusion: In all, though most allo-HSCT had mild symptoms from H1N1-infection, heavy immunosuppression and emergence of oseltamivir resistance were likely responsible for a substantial morbidity, further highlighting the need for vaccination and monitoring of close contacts and family households, especially children.

P760
High titres of pre-existing adenovirus serotype-specific neutralizing antibodies predict viral reactivation after allogeneic stem cell transplantation in children
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Objectives: Human adenovirus (HAdV) infections are frequent in children after allogeneic stem cell transplantation (SCT) and may have a fatal course. Whether these infections occur through reactivation of endogenous virus or are transmitted via the graft remains a matter of debate.
Methods: In a cohort of 24 pediatric patients who received SCT, 35 infections with one or more of 5 serotypes of HAdV, i.e. 1, 2, 5, 6 and 31, were detected by culture. Serum titers of serotype-specific neutralizing antibodies (NAb) against these 5 serotypes of HAdV were measured before transplantation. Graft material was analyzed for the presence of HAdV DNA.
Results: High titers of NAb against a certain serotype in the recipient prior to SCT, likely reflecting previous infection, appeared to predispose for infection with the same serotype after SCT, instead of conferring protection. Because in only one case of 41 samples of graft material, a very low level of HAdV DNA was detected, it is unlikely that HAdV is transferred from the donor.
Conclusion: Together, these data suggest that adenoviral complications after SCT are caused by reactivation of endogenous persistent HAdV rather than by de novo infection from donor or environment. This observation may offer a strategy of prophylactic treatment of high risk patients before SCT to prevent infectious complications after allogeneic SCT.

P761
Prior invasive mould infections are not a major risk factor for death in patients who undergo allogeneic haematopoetic stem cell transplantation
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Background: Advances in allogeneic HSCT over the last decade have reduced organ damage, infection and acute GVHD. With improved diagnostics and antifungal treatment, we hypothesized that invasive mold infections (IMIs) prior to allo-HSCT would no longer represent a significant risk factor for patient death after transplant.
Methods: We identified AML or MDS patients who developed an IMI documented by MSG/EORTC criteria prior to allogeneic HSCT at M.D. Anderson Cancer Center during 2005-2008. We collected data on the transplant procedure and complications, co-morbidities and infections. Variables screened by univariate analysis were fitted to a multivariate regression models to assess independent risk factors associated fungal relapse and survival.
Results: 60 patients were identified (52 AML, 8 MDS). Most had active (30%) or refractory (53%) leukemia at the time of allo-HSCT and received mold-active secondary prophylaxis (54%). Most IMIs were not microbiologically documented (58%). The most common documented IMIs were Aspergillus (23%), Mucorales (8%), Curvularia (3%) and Fusarium (2%). Treatment for the IMI prior to transplant included combination (66%); single agent (29%) or triple drug regimens (5%), which consisted of triazole-echinocandin combinations (28%); lipid amphotericin B (LAMB)-echinocandin (23%), LAMB monotherapy (17%), LAMB-triazole (15%), or triazole montherapy (10%). Most patients had complete (62%) or partial (15%) response to treatment prior to transplant, with fewer patients exhibiting evidence of stable disease (13%) or progression (7%) on therapy. Most patients with prior IMIs (73%) did not develop a recurrent or new infection after transplant, whereas 12% of patients relapsed, 10% failed secondary prophylaxis, and 5% developed a breakthrough infection. Risk factors for recurrent infection included poor response of initial IMI (P=0.03) and TNF-α inhibitor therapy (P=0.001). Secondary prophylaxis modestly reduced the risk of recurrent IMI (P=0.07). Survival at 6 months (64%) and 1 year (57%) was similar in all patient groups. Status of the underlying malignancy was the most important factor influencing 1 year survival (OR 1.5; 95% CI 0.94-2.5; P=0.08).
Conclusions: Most patients with prior IMIs did not develop evidence of relapsed IMI following allogeneic HSCT, especially if they had good response to initial treatment. Our data suggest that prior IMI should only be considered a “soft contraindication” to subsequent allo-HSCT.

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BK virus infection in paediatric stem cell transplant recipients - does it matter?
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Polyoma BK virus (BKV) associated haemorrhagic cystitis (HC) is a severe complication following allogeneic stem cell transplantation (SCT).
BKV was screened weekly between day -10 until day +100 by quantitative PCR in urine and peripheral blood (PB) in 92 consecutive pediatric allogeneic SCT recipients. Median age was 8.3 years; 61 patients underwent SCT for malignant and 31 patients for non-malignant diseases. Conditioning regimen was myeloablative in 53 and reduced intensity in 39 cases.
Urinary excretion of BKV was detectable in 42 patients (46%) with a median viral load of 10^5 (range 10^2 – 10^{11}) per ml. In 14% of cases BKV was also detectable in PB (median viral load 10^3/ml, range 10^2-10^5). Thirteen/19 (68%) cases with a viral load in urine >1x10^8 developed viremia in contrast to 0/79 cases with a viral load below 10^8 (p< 0.0001). Ten out of 19 (53%) patients with a viral load in urine >1x10^8 developed HC. HC was associated with a viral load >10^8 in urine in all cases.
Overall BKV infection did not correlate with donor-type (matched sibling 50%, matched unrelated 64%, haploidentical 40%), conditioning regimen (myeloablative 57%, reduced intensity 31%), T-cell depletion (61% without, 43% with T-cell depletion) and graft-versus-host disease (GvHD grade 0-II: 65%, grade III-IV: 53%), however in none of 13 transplants without in-vivo or ex-vivo T-cell depletion BKV was detectable in PB.
Virus associated transplant related mortality (TRM) was observed in 4/37 (11%) cases without BKV infection, 3/41 (7%) cases with BK-viruria and 5/13 (38%) patients with BK-viremia (p=0.003).
BKV shedding in urine is frequently detected following SCT but is associated with HC in only 24%. However, a high viral load in urine is a significant risk factor for HC in children after SCT. Quantitative PCR may be a helpful tool concerning preemptive treatment strategies.

BK-viremia seems to reflect the degree of overall immunosuppression due to either T-cell depletion or GvHD.

P763

Comparison of EBV viral load in plasma, buffy-coat and whole blood of haematopoietic stem cell transplant recipients at risk of developing post-transplant lymphoproliferative disease

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The monitoring of EBV viral load is recommended in HSCT recipients at risk of developing post-transplant lymphoproliferative disease (PTLD). However, due to the lack of standardization of PCR techniques, many questions remain unanswered.
Methods: EBV viral load was retrospectively assessed in plasma (PL), buffy coat (BC) and whole blood (WB) of 549 blood samples from a cohort of 50 HSCT recipients. Inclusion criteria were unrelated/mismatched transplants or T-cell depletion or ATG/OKT3 use. EBV DNAemia was assessed weekly by an in-house quantitative real time PCR from conditioning to day +120.
Results: Kappa index (K) showed moderate agreement in PCR positivity between WB/BC samples (K=0.52), and fair agreement between PL/BC (K=0.33) or PL/WB (K=0.23) samples. Two patients (5.3%) were EBV seronegative before HSCT and remained so during follow-up. Thirty-eight (79.2%) of the remaining 48 recipients presented EBV reactivation. The frequency of EBV reactivation varied according to the type of sample tested, being 56.3%, 64.6% and 70.2% in PL, BC and WB, respectively. Median time to reactivation was 33 (-9 to 135) days in WB and BC samples, and 59 (2-109) days in PL samples. Proven or probable EBV disease was diagnosed in 7 of the 38 pts (18.4%) with EBV DNAemia. Higher EBV viral load was not significantly associated with the severity of EBV disease. Ten out of 38 patients (26.3%) who reactivated died within the first 50 days of EBV reactivation.
Conclusions: EBV reactivation is frequent after unrelated donor or mismatch related donor HSCT. EBV viral load in WB samples detects the highest number of episodes of EBV reactivation and allows preemptive reduction in immunosuppression, if possible, and/or prompt introduction of rituximab. An international standard for EBV quantification is urgently needed.

P764

Use of rituximab in the treatment of Epstein-Barr virus reactivation after allogeneic stem cell transplantation: a retrospective study in 86 patients

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Background: Allogeneic stem cell transplantation can induce an Epstein Barr Virus (EBV) reactivation. This risk increases with unrelated donor transplantation, advanced age, mismatch graft, use of anti-thymocyte globulin (ATG). While, there is no treatment guideline, the use of rituximab is usually reported.
Objective: To assess the efficiency and tolerance of rituximab, we have conducted a retrospective study between 2005 and 2009 in allograft unit of University Hospital in Montpellier. We have included all patients with a viral load >500 copy/ml and receiving rituximab (375 mg/m²/week) until negative viral load. Data processing was performed using STATS GRAPHIC software. Quantitative variables, not following a Gaussian distribution, were compared by nonparametric tests.
Results: Among the 86 patients with EBV reactivation, 29 (34%) received myeloablative regimen, 57 reduced intensity regimen. Mean of age was 47 Years (±13.5). 47 reactivations (68%) occurred with unrelated donor transplantation. 30 (33.4%) concerned mismatched allografts. 48 (56%) patients have received ATG. 30 (35%) patients reactivated another herpes virus, at the same time.
Most of reactivations occurred before Day 100 with a median Day 165 (5-1531). In 79 (92%) cases, couple recipient-donor had a positive EBV serology before allograft, in 4 cases (5%) the recipient only was positive. 51 (59%) patients reached complete response, 26 (30%) partial response. The delay for response was 4.4 weeks (±2.4) and could be shortened with a simultaneous use of ganciclogir for CMV. The relapse rate after end of treatment was 45%. Two patients developed a post-transplant lymphoma disorder. The pathology, kind of donor, regimen, match, chimerism, use of ATG did not influence time to response. Rituximab decreased rate and severity of acute Graft versus Host Disease (GvHD) ($p<0.01$). Rituximab did not modify TCD4 and TCD8 lymphocyte count. NK cell increased start at 6 months after the end of treatment, B lymphocytes from 3 to 6 months. Median overall survival (OS) was not reached at 2 years. The deaths related to EBV occurred earlier than those bound to another cause (50 days vs 366 days, $p<0.01$).
Conclusion: Rituximab is efficient and safe to treat EBV reactivation and could erase factors of poor prognosis. Furthermore, the use of rituximab for EBV reactivation could bring a profit on acute GvHD.

P765

Optimized molecular diagnosis of invasive aspergillosis in patients after allogeneic stem cell transplantation – a second confirmatory assay is crucial

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Objectives: Invasive aspergillosis (IA) is still a major complication in immunocompromised patients after allogeneic stem cell and solid organ transplantation. Mortality rate is high and reaches up to 100% in cerebral IA. Diagnosis of IA remains difficult because of unspecific clinical signs and insensitivity of conventional fungal diagnosis. Therefore, early and sensitive molecular assays are highly warranted. In our study, EDTA whole blood and serum specimens were collected twice weekly from October 2008 until October 2010 for comparative analyses, followed by defined DNA extraction and PCR detection protocols. Comparison of inter-assay performances, sensitivities and variability leads to optimization of molecular systems used to detect IA.
Methods: Patients after allogeneic stem cell transplantation were categorized according to the criteria for IA of the European Organization for Research and Treatment of Cancer/Mycoses Study Group. Whole blood samples were prospectively analyzed for the presence of Aspergillus-DNA by an in-house DNA extraction method followed by qPCR (ITS) and in parallel by Platelia Aspergillus EIA (Biorad; GM). Additionally, selected sera from patients with probable IA (n=10) and control episodes (n=9) were extracted using the QIAamp UltraSens Virus kit (Qiagen), followed by our in-house PCR assay (SEP).
Results: We collected 3595 blood samples from 280 high-risk patients. From these, 19 patient episodes were selected and analyzed again by ITS, GM and SEP in parallel.
Single positive results in controls were obtained by ITS, GM and SEP in 67%, 11% and 33% (197 samples), and in 100%, 90% and 90% of the probable IA patients (189 samples), respectively. Reanalysis of the data (a first positive result had to be confirmed by a second positive within 10 days) revealed that the combinations GM/SEP, ITS/GM and ITS alone ranked best detecting 90%, 80% and 70% of the probable IA cases, respectively. By ITS, we revealed a positive PCR result in 6/10 patients prior to the EORTC criteria for probable IA, whereas this occurred in only 4/10 cases by GM and SEP, respectively.

Conclusions: Whole blood analysis by ITS showed high sensitivity, but low specificity. Therefore, we recommend performing GM or SEP in parallel allowing confirmation of a single positive result by a second diagnostic assay within 10 days, resulting in a significant increase in sensitivity and specificity.

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Respiratory virus infections other than influenza virus in allogeneic stem cell transplant recipients

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Background: Respiratory virus infections following allogeneic stem cell transplantation (alloSCT) have been sometimes associated with mortality and morbidity. We retrospectively analyzed clinical features of respiratory virus infections of alloSCT recipients in our institute.
Methods: Oropharyngeal swab, sputum, or bronchoalveolar lavage fluid were sampled from alloSCT recipients with respiratory symptoms. Respiratory viruses were detected by viral culture or RT-PCR. Respiratory virus infection was diagnosed by both the presence of respiratory symptoms and detection of respiratory virus. Upper (URTI) and lower respiratory tract infection (LRTI) were diagnosed by radiologic findings.
Results: We analyzed 530 recipients undergone alloSCT in our institute from January 2006 to December 2010. Sixty-one patients were diagnosed as respiratory virus infection. The viruses detected included respiratory syncytial virus (RSV, 22 cases), parainfluenza virus type 3 (PIV3, 37 cases), and adenovirus (ADV, 4 cases). In one patient, RSV and PIV3 were detected in separated periods. LRTI by RSV, PIV3, and ADV developed in 13 (59%), 10 (27%), 3 (75%) cases, and their mortality rates were 61%, 50%, 66.7%, respectively. The co-infection of other pathogens (Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Aspergillus spp., et al) was more highly associated with the mortality of RSV LRTI than those of PIV3 or ADV LRTIs. Risk factors for death by respiratory virus infection were low lymphocyte counts (Lymphocyte <200/μL), LRTI, and onset before neutrophil engraftment. And, although patients with respiratory virus infection were isolated immediately after the symptom developed, RSV and PIV3 caused outbreak in our transplant unit.
Conclusion: Respiratory virus infections at early post-transplant period were associated with high mortality rate. Respiratory viruses also caused outbreak in transplant unit. Considering that effective treatments for these viruses have not been established, more rigorous preventative strategy, especially for those in high-risk group, is extremely important.

P767

Analysis of efficiency and costs of antifungal treatment in patients undergoing allogeneic haematopoetic cell transplantation: "real life" evaluation

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Background: Antifungal prophylaxis (AP) and therapy (AT) are increasing the costs in patients (pts) undergoing alloHCT, who are at high risk to acquire invasive fungal infections (IFI). Efficiency of AP and diagnostic procedures still need to be improved and evaluated.
Patients and methods: We perform a prospective study analysing all pts undergoing alloHCT in 2010, in terms of HCT-related data, frequency and costs of AP/AT, choice and costs of the applied diagnostic procedures and pre-emptive or empiric therapy. Further, toxicity of AP/AT and breakthrough IFI is documented. All pts. receive CT scan of the lung before HCT, after engraftment and in case of respiratory insufficiency. Galactomanan (Gal) and ß D-glucan (ß-D) tests are performed every week till engraftment.
Results: Up to now 83 pts. median age 58 y (20-73) are evaluated; the underlying disease is AML/MDS (n=43), lymphoma (n=23), MPS (n=11), ALL (n=6), transplanted mostly with advanced disease (n=58). At admission 19 pts. had probable/possible IFI and received standard AT. All other pts received AP mainly fluconazol (FLU) 200mg (n=44) or in case of IFI in the past liposomal amphothericin B (LAMB) 1mg/kg (n=16), voriconazol (VOR) 400 mg (n=1) or posaconazol (POS) 600mg (n=3). After alloHCT in case of suspected IFI preemptive or empiric (which comes first) treatment consisted of LAMB 3 mg/kg (n=38), VOR 4 mg/kg bid (n=32), caspofungin (CAS) 50 mg (n=16) or POS 800mg (n=3). Overall n= 34/64 had no change between AP and end of evaluation (n=30/44 of FLU, n=4/16 of LAMB); n=30 (44%) had a suspected or possible IFI (14/44 of FLU; 12/16 of LAMB, 3/3 of POS, 1/1 of VOR). Preemptive or empiric AT was initiated or changed (n=27) because of: positive CT (n=35), pos. GAL (n=12), pos. ß-D (n=13) or fever despite antibacterial therapy (n=23). Median cost /pt. were: 220 € for CT scan, 159 € ß-D and 90 € GAL. All grade toxicity was observed for liver (n=72) and kidney (n=76). Mean costs of drugs and diagnostics were 6047 €/pt. Overall, despite AP, 44% had a suspected breakthrough IFI. No pt. died due to IFI.
Conclusion: Antifungal prophylaxis and therapy are the major causes for high expenses in alloHCT. Our "real life" analysis once again highlights the importance of efficient concepts for diagnostic, AP and AT especially in pts. receiving alloHCT. Data of all pts. of 2010 (n=107) with detailed results will be presented. This prospective evaluation project is supported with a grant by GILEAD.

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Immune reconstitution is influenced by the number of CD34+ cells in the transplant material and further shaped by Herpes viruses infection/reactivation

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Successful outcome of HSCT depends largely on the immune reconstitution post transplant. To evaluate the pace of immunological recovery the lymphocyte profiling post-HSCt was conducted in one week intervals starting at the beginning of the hematological reconstitution. The study included 44 patients (age 1 to 64, 21/23 RIC/MAC, 21/23SIB/MUD, BM/PBPC 4/40. The following methods were employed:

1. Cytometry for detection CD34+, CD4+, CD8+, CD20+; CD4+ cells, the latter evaluation included enumeration of Naive cells (NV) CD4+CCR7+CD45RA+, Central memory (CM) CD4+CCR7+CD45RA-, Effector memory(EM) CD4+CCR7-CD45RA-, Terminally differentiated memory (TEMRA) CD4+CCR7-CD45RA+ lymphocytes.
2. For biological assessment of the immune competence Herpes virus(CMV,EBV,HHV-6) reactivation/infection was surveilled with the use of the Real – Time PCR.

It was found

1 At the beginning of hematological recovery:
   1.1. The number of CD4+ NV cells in blood was associated with the number of CD34+ cells in the transplant material (2,45 vs 8,31 cells/ul, p=0,025, in patients receiving below and above the median value of CD34+ cells in transplant material of all patients).

1.2. The low number of CD4+ (median 44 cells/ul), CD4+ EM (22cells/ul) and CD8+ (51 cells/ul) lymphocytes were predictive of CMV reactivation observed two to three weeks later.

2. After hematological recovery:
   2.1. In all HHV-6 and EBV positive cases CD4+ and CD20+ lymphocyte increase was seen at the reactivation, respectively.
   2.2. HHV-6 infection resulted, as seen in 2–3 weeks after reactivation observations, with the lowest number of CD4+ blood lymphocytes (93 cells/ul vs 190 cells/ul in patients with and without HHV-6 reactivation (p=0,044, U-test). HHV-6 positive patients had poorer one year survival as compared to those lacking HHV-6 copies in blood (56% vs 75%).
   2.2. CMV infection resulted in an increase of CD8+ lymphocytes in blood (530 cells/ul vs 190 cells/ul U test, p=0,01) and that of EBV with an increase of CD20+ lymphocytes in blood (18 vs 8 cells/ul, p=0,07).

In conclusion: CD34+ cells content in the transplant material influences the immunological recovery. Biologically CMV, HHV-6 and EBV reactivation associates with an increase in blood CD8+, CD4+ and CD20+ lymphocytes, respectively. HHV-6 reactivation had a strong impact on CD4+ lymphocyte number what associates with poorer survival of HHV-6 positive patients.

P769

GMP-grade generation of B-lymphocytes for adoptive immunotherapy in patients after allogeneic stem cell transplantation

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Background and objectives: We have recently shown that memory B-lymphocytes from murine CMV immune donor animals adoptively transferred into immunodeficient mice were highly effective in protecting from a viral infection indicating a therapeutic potential of virus specific memory B-cells. These preclinical data provided evidence that a cell-based strategy supporting the humoral immune response might be effective in a clinical setting of post-HSCT immunodeficiency (Klenovsek et al., 2007, Blood 110: 3472-9). As adoptive transfer of B-cells has not been used before in a clinical setting, it is necessary to establish a technology for the generation of GMP-grade B-cell products.
Methods: Starting from the leukapheresis product of healthy blood donors, B-cells were purified by two different separation strategies using GMP-grade microbeads and the CliniMACS™ device. A one-step protocol was used for positive enrichment of B-lymphocytes with anti-CD19 microbeads. In a two-step enrichment protocol, first T-lymphocytes were depleted by anti-CD3 microbeads and the remaining fraction was positively selected by anti-CD19 microbeads.
Results: The leukapheresis products contained a mean of $9.0x10^8$ CD19-positive B-cells (range $4.5\text{-}12.4 x10^8$). After the one-step positive purification strategy a mean purity of $CD20^+$ B-lymphocytes of 78.1% with a recovery of 32-41% was obtained. With the two-step T-cell depletion/B-cell enrichment protocol we achieved a mean purity of 96.4% (range 93.4-97.8%) with a slightly lower recovery of 14-37%. The absolute B-cell numbers obtained in the product was 1.3 to 4.0 $x10^8$ and 1.7 to 2.6 $x10^8$ for the one-step positive enrichment and the two-step protocol, respectively. Importantly, the absolute number of T-cells was lower in cell products after the two-step protocol (0.1 to 0.9 $x10^6$ T-cells) as compared to the one-step positive CD19-enrichment (1.6 to 3.4 $x10^6$ T-cells). Assuming a patient with 70 kg bw, the B-cell products obtained after the combined CD3-depletion and CD19-enrichment contained less then $4x10^4$ T-lymphocytes/kg bw, which is a critical threshold number of T-cells in haploidentical HSCT. The B-cell products showed an antibody production after in vitro stimulation and showed excellent viability after cryopreservation.
Conclusions: A GMP-grade B-cell product can be obtained with high purity and very low T-cell contamination using the two-step enrichment protocol based on CliniMACS™ technology. (Supported by BayImmuNet.

P770

Vaccination against H1N1 in patients after allogeneic haematopoietic stem cell transplantation: results from a retrospective analysis

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Background: For the 2009 H1N1 pandemic an AS03-adjuvanted vaccine (Pandemrix®) was used in Germany. Serologic responses have not been evaluated previously in recipients of allogeneic hematopoietic stem cell transplants (alloHSCT).
Methods: In 2009 serologic responses to either adjuvanted H1N1 vaccine (n=36) or H1N1 infection (n=2) after alloHSCT were studied in 38 HSCT recipients. Fourteen were vaccinated twice. Responses were measured with a standard hemagglutination-inhibition (HI)-assay, seroconversion and seroprotection rates were calculated. Median time between vaccination and serum collection was 35 days (range: 14-70). The average patient age was 48 years (range: 18-68). Median time between HSCT and vaccination was 453 days (range: 111-2893). While none of the patients had active acute graft versus host disease (aGvHD) at the time of vaccination/infection, 21 patients (55%) had chronic GvHD (cGvHD) at any time and 14 patients (37%) had active cGvHD (mild (n=10), moderate (n=5) or severe (n=1)) at time of vaccination/infection. While 11 patients had no immunosuppressive therapy, 13 had mild, 11 moderate and 3 intense immunosuppression. Seven patients received treatment with rituximab for cGVHD before vaccination.
Results: Nineteen patients (53%) responded to H1N1 vaccination. Two patients had H1N1-infection without prior vaccination and one patient severe H1N1-infection with ARDS despite prior single vaccination. Previous treatment-failure to primary therapy of aGvHD was associated with failure to H1N1 vaccination. Non-responders to vaccination more often had cGvHD (65% versus 53%) and received second or third line therapy (53% versus 11%), while responders mostly had first line therapy for cGvHD. While vaccination responders had no (n=9, 47%) or single agent immunosuppressive therapy (n=5, 26%), non-responders frequently received moderate or intense immunosuppression, as 8 patients (47%) received combination treatments und 6 patients (35%) were treated with rituximab. None of the patients receiving rituximab responded to vaccination.
Conclusions: The overall response to H1N1 vaccination in HSCT recipients was modest. Response rates were higher in patients without immunosuppressants and in cGvHD patients under single agent immunosuppression. Patients under combined immunosuppressive therapy for cGvHD hardly responded to H1N1 vaccination and rituximab treatment completely abrogated H1N1 responses.

P771

Mannose-binding lectin ELISA is a new approach to predict the chance of infectious complications during autologous haematopoietic stem cell transplantation

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Haematopoietic stem cell transplantation causes immuncompromised state and high risk of infectious complications.

Gram-positive cocci are responsible for the majority of the post-transplant bloodstream infections. Viral and invasive fungal infections can be significant causes of morbidity.
Mannose-binding lectin (MBL) is involved in innate immune response. MBL binds microbial surface carbohydrates and mediates opsonophagocytosis. MBL also functions as co-receptor of Toll-like receptor. MBL deficiency is a result of impaired assembly or stability of multimers.
In patients who received high dose chemotherapy, the innate immundeficiency is an other risk factor of infectious complications.
According to literature, significant association was shown between low concentrations of MBL and serious infections. Infections can cause delay of engraftment after transplantation.
In our study we investigated the association between serum MBL level and frequency, severity and occurance of infections. We formed subgroups, i.e. multiple myeloma, non-Hodgkin and Hodgkin lymphoma and we compared the infectious complications.
A double-monoclonal antibody sandwich ELISA system (BioPorto, Denmark) was used, which is a sensitive method for determining the MBL antigen levels in the sera.
We measured 79 patients' MBL levels in a retrospective study, after transplantation in the absence of active infection. The range of MBL level in healthy population is between 5 and 5000 ng/ml, <100 ng/ml is defined as MBL deficiency. 7 patients were MBL deficient. The median time of the onset of first infection after transplantation was the 5th day in MBL deficient, while the 16th day among non-MBL deficient patients. There were more infections among MBL deficient patients (2,57 vs 2,06 infection episodes/patient). When patients with more and less than 500 ng/ml serum MBL level were compared, similar trends were seen, but the difference was not significant. The occurance of absolute MBL deficiency was not different between patients with malignant haematological diseases and healthy controls (9% vs 14%). MBL level was the highest among multiple myeloma patients.
MBL deficiency may predispose to infections. MBL deficient patients have a greater number of severe infections and experience their first severe infection earlier, compared to nondeficient patients. The measuring of MBL may be helpful in antibiotic treatment, in case of MBL deficiency earlier and more intensive treatment may be indicated.

P772

Therapeutic granulocyte transfusions in neutropenic patients with invasive pulmonary aspergillosis and haematological malignancies

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Background: Granulocyte transfusion (GTX) is used as an additional therapeutic option in patients with severe neutropenia following chemotherapy constituting an increased risk for life-threatening bacterial and fungal infections. We hypothesized that interventional GTX would provide a clinical benefit for neutropenic patients with invasive pulmonary aspergillosis (IPA).
Methods: We reviewed the clinical outcome of 44 patients with severe neutropenia (46 cases) and underlying haematological malignancies suffering from IPA unresponsive to standard antifungal therapy who received a total of 181 human recombinant granulocyte colony-stimulating factor (rh G-CSF) stimulated GTX at Freiburg University medical center from 1996–2009. Fourteen patients (32%) received GTX after allogeneic and 4 (9%) after autologous hematopoietic cell transplantation (HCT). Donors were exclusively relatives and acquaintances of the recipients. Diagnosis of IPA was achieved by computed tomography (CT) scan or serological or microbiological detection. Response of GTX on infections was confirmed by repeated CT, decreasing C-reactive protein (CRP) levels, hematopoietic regeneration and clearance of aspergillus antigen on serial measurement.
Results: A median of 3.9 GTX (range 1-25) containing a median total of 5.83x10e10 (range 0.3–11.28x10e10) white blood cells per GTX were administered. All but four (2%) transfusions were well tolerated. Mean duration of neutropenia proceeding GTX was 20 d (2-70). Resolution of infection or clinical improvement was achieved in 29 (63%) patients with IPA and haematopoietic recovery has been assumed within five days after the last GTX in 21 patients (46%). Thirtythree (72%) patients were alive one month after the last transfusion and median survival was 183 days. Overall, progressive malignant disease was the main cause of death. Patients who did not respond to GTX died without exception on septic complications despite appropriate antibacterial and antifungal treatment. Nine out of 26 neutropenic patients receiving GTX after conventional chemotherapy underwent allo-HCT later on after control of IPA.
Conclusions: Rh G-CSF stimulated GTX is a safe and effective therapeutic tool for patients with haematological malignancies and antifungal-resistant IPA following severe neutropenia after chemotherapy. GTX may serve as an antifungal bridging therapy in severe neutropenic patients with IPA scheduled for allo-HCT.

P773

Detection of human herpesvirus-6 in biopsies from patients with severe gastrointestinal symptoms after allogeneic stem cell transplantation

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Gastrointestinal complications are frequent side effects after allogeneic stem cell transplantations. Main differential diagnoses of diarrhoea and vomiting are acute GvHD, viral or bacterial infections and toxicity of the preparative regimen. Particularly viruses of the herpes family are often the causative agent for GI-symptoms. Viraemia of human herpesvirus-6 (HHV-6) can be observed frequently after allogeneic stem cell transplantation. The pathogenic significance of this finding is still not known.
Patients: In this study 51 patients after allogeneic stem cell transplantation with severe vomiting or diarrhoea were retrospectively analyzed. 102 biopsies obtained by colonoscopy or endoscopy of the upper gastrointestinal tract were analyzed histologically for signs of GvHD and by PCR for viral DNA of HHV-6 and other virus of the herpes family.
Results: DNA of HHV-6 was detected in 38 of 75 initial samples (51%) and in 19 of 27 follow-up biopsies (70%). In the initial specimen HHV-6 was equally distributed in the presence or absence of histologically proven acute GvHD (54% vs. 47%). At the time of the first endoscopic investigation patients received either antiviral prophylaxis with aciclovir (68%) or antiviral therapy by ganciclovir, aciclovir or foscarnet (32%). After detection of HHV-6 nine patients received antiviral therapy. As antivirals patients received either high-dose intravenous aciclovir (3x10 mg/kg, n=3), foscarnet (3x60 mg/kg, n=4) or a sequential therapy with ganciclovir (2x5 mg/kg) and foscavir (n=2) according to the patient's immune status and concomitant viral infections. Despite high dose antiviral therapy no patient with initial positive HHV-6 PCR turned negative in follow up biopsies after treatment. By univariate analysis a graft from an unrelated donor was the only risk factor for HHV-6 infection with a trend to significance (p=0.09). Patients with detectable HHV-6 in GI-samples had a higher rate of transplant-related mortality compared with patients with negative biopsies (34% vs. 16%). However, there was no difference in overall survival between patients with or without HHV-6 detection in the gastrointestinal tract. Conclusions: HHV-6 is a frequent finding in biopsies of patients with severe gastrointestinal symptoms with or without acute GvHD after allogeneic stem cell transplantation. Further studies are needed to define the pathogenic role of HHV-6 and to evaluate efficient antiviral treatments.

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Parenteral colistin is relatively safe for patients with haematological malignancies and HSCT recipients

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Introduction: The incidence of multidrug-drug resistant (MDR) Gram negative rods infection among patients with hematological malignancies and hematopoietic stem cell transplantation (HSCT) recipients is increasing. Colistin is among the few therapeutic options. However, its potential nephrotoxicity is of concern, especially as nephrotoxic medications are frequently administered concomitantly.

Methods: Data on patients with leukemia, lymphoma and those undergoing HSCT treated for more than 48 hours with intravenous colistin (2.5–5 mg/kg/day in children and 240-480 mg/day in adults, adjusted to renal function) during 2008-2010 in Hadassah University Hospital was retrospectively collected and analyzed. Nephrotoxicity was defined as a 50% increase in serum creatinine over the normal range, as compared to pre-treatment levels, during colistin administration and 2 days after its discontinuation.

Results: 20 patients aged 0.5-70 (median 38) years received 28 courses of intravenous colistin for 3-28 (median 10) days. Underlying conditions included allogeneic HSCT (14 courses), acute myelogenous (8), acute lymphocytic (1), and chronic lymphocytic leukemia (1), and lymphoma (4). The indications for treatment were bacteremia with MDR Klebsiella pneumoniae (4), Pseudomonas aeruginosa (2), and Escherichia coli (1); 22 courses of colistin were given as empirical therapy for nosocomial clinical sepsis, 3 of which were for pneumonia. During 23 (82%) courses nephrotoxic medications were administered concomitantly, including one or more of the following: vancomycin (12), acyclovir (12), aminoglycosides (9), cyclosporine (7), ganciclovir (3), amphotericin (3), foscarnet (2). Creatinine levels were normal at the start of 26/28 (93%) courses. Nephrotoxicity was observed in only 3 (11%) courses within 5-14 days of colistin therapy. Two of these patients died of infectious complications and in the third patient renal function recovered 2 weeks after colistin discontinuation. Two patients had convulsions, probably not related to colistin. No other side effects were seen.

Conclusion: Treatment with parenteral colistin that is adjusted to renal function was found to be relatively safe for children and adults with hematological malignancies or following HSCT even with concomitantly administered nephrotoxic medications. Renal function monitoring during colistin therapy is mandatory.

P775

Video-assisted thoracoscopic biopsy is feasible and safe in adult haemato-oncology patients undergoing stem cell transplantation or high-dose chemotherapy

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Objectives: Invasive Aspergillosis (IA) is a serious but difficult diagnosis in patients undergoing high dose chemotherapy or haematopoietic stem cell transplantation (HSCT). For clinical trials the European Organization for Research and Treatment (EORTC) revised criteria is a useful diagnostic tool but there is little data on its usefulness in clinical practice. We set up an observational prospective cohort study in order improve our diagnostic and management strategies using the EORTC/MSG criteria as a diagnostic tool. Here we looked at the feasibility and safety of tissue diagnosis using video-assisted thoracoscopic (VATS) biopsy in patients with suspected IA.

Methods: All study patients were prospectively recruited and followed up for≥4 months after chemotherapy or HSCT. During inpatient admission twice weekly serodiagnostic surveillance was performed and neutropenic sepsis unresponsive to second-line antimicrobials triggered diagnostic work up for IA including computerised tomography (CT). Patients with suspected lesions on CT were referred for VATS biopsy. Thirty one patients underwent biopsy between 26/3/2009 and 26/11/2010.

Results: A total of 38 biopsies was performed on 31 patients. One was excluded due to lack of histology. The median age was 34 years (range 21-73y) with equal male/female ratio and the median (range) follow-up was 205 (115-476) days. Fifteen patients (49%) had myeloid malignancies, 10 (32%) had lymphoid malignancies and 6 (19%) had aplastic anaemia. At the time of biopsy the treatment received was none in 1 (3%), immunosuppressive therapy in 4 (11%), chemotherapy in 9 (24%), autologous HSCT in 5 (13%), and allogeneic HSCT in 18 (49%). The median (range) pre-biopsy parameters were: platelets 120 (45-399), haemoglobin 9.5 (6.9-13.1) and neutrophils 1.4 (0.02-7.35). The biopsy was a VATS procedure in 32 (86%) cases and 4 of these involved pre-surgical CT-guided methylene blue targeting. The results of the biopsy was helpful in 14 (38%) where IA was proven in 4 cases while in 12 others an alternative diagnosis was made. In the remaining 23 cases non-specific inflammatory features were present in 20 and technical difficulties in 3 resulted in failed biopsies. The procedure was well tolerated in all patients with no mortality and limited morbidity (pain and minor pneumothorax) and no bleeding.

Conclusion: VATS biopsy is a safe and useful diagnostic tool which could help guide clinical decision making and reduce unnecessary treatment.

P776

Normalized EBV quantity in patients after allogeneic haematopoietic stem cell transplantation

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Objective: EBV remains important pathogen in patients after haematopoietic stem cell transplantation (HSCT). Beside benign reactivation, EBV can cause localised or generalised form of EBV associated lymphoproliferative disease (EBV-LPD) with clinical symptoms such as fever, hepathopathy, lymphoma-like symptoms and generalised B cell proliferation in peripheral blood.

Methods: Between I/2002 and XII/2010, we tested prospectively whole blood samples from 269 children (8137 samples) and from 500 adults (7446 samples) after allogeneic HSCT. A minute number of additional tissue and body fluids samples were tested too. Retrospectively, we tested 50 samples from 4 children who died for EBV-LPD in year 2000. EBV and albumin gene were quantified using RQ-PCR and quantity of EBV was normalised to 100000 human genome equivalents.

In the prospective testing, EBV was detected in whole blood of 200 children (74.3%; 2571 samples; median 90 normalised viral copies (NVCs)) and from 308 adults (61.6%; 1807 samples; median 80 NVCs). More than 1 000 copies were detected in 69 children and 64 adults, more than 10000 in 19 children and 17 adults. Clinical or laboratory signs of EBV-LPD were observed in 7 children and 9 adults. Proved generalised EBV-LPD was detected in 6 patients (mean of highest EBV 107005 NVCs), localised form in 2 child and 5 adults (mean of highest EBV 56550 NVCs). Successful treatment of EBV-LPD with rituximab was used in 6 children and 3 adults; EBV crossed 10000 NVCs in all of them. All the patients with generalised form were successfully treated but only one patient with EBV+ Burkitt lymphoma of donor origin developed and one with Diffuse large

B cell lymphoma were subsequently successfully treated with chemotherapy. Among retrospectively tested patients, highest quantity was between 1165000-11060500 NVCs and the interval between the first detection of EBV and decease was 24-91 days.
Conclusions: EBV can be detected in big proportion of patients after HSCT, more frequently in paediatric population. Level of 10000 NVCs seems to be a good level for possible treatment and it is 100 times lever than highest quantity detected patients who died of EBV-LPD. Long lasting EBV positivity in patient with Burkitt lymphoma suppose to be EBV released from the local proliferation. Normalisation proved to be useful approach for comparison of EBV levels in the whole blood and in the tissues.
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P777

A study of prognostic factors affecting the incidence and outcome of invasive fungal infections occurring in neutropenic and allogeneic transplant patients receiving prior antifungal agents

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Mold-active antifungal agents are useful for the prevention or treatment of suspected fungal infections in patients with hematologic malignancies. However they may fail due to breakthrough invasive fungal infections (B-IFI). Several host and antifungal related factors may underlie this lack of efficacy. In this retrospective study we aimed to identify the risk factors for incidence and outcome of B-IFI. Definitions were based in 2008 EORTC/MSG criteria. Outcome was recorded by response and survival at the end of antifungal treatment and after 90 days.
We recorded 62 consecutive cases of B-IFI in neutropenic or allogeneic transplant patients who were receiving mold-active antifungal agents and 122 controls matched for disease and type of treatment from the same institutions. Their main characteristics are shown in Table 1. Cases had a higher median age (p=0.009). Disease diagnosis, status and allogeneic transplants were well balanced. More cases had acute GVHD (p=0.039). The use of antifungal agents was not uniform. Prophylaxis was azol-based in 62,2% and 88,5% of cases and controls, respectively. Empiric therapy was based in equinocandins in 56,9% in both groups, and pre-emptive therapy equally distributed (50%) between liposomal amphotericin B and azol. There were 37 (59.7%) and 25 (40.3%) cases of probable and proven B-IFI. Overall, 29 (46.8%), 22 (35.5%) and 11 (17.7%) cases occurred after prophylaxis, empiric and pre-emptive antifungal treatment. Long-lasting neutropenia (p=0.0002) and acute GVHD (p=0.039) were associated with B-IFI. Proven B-IFI were due to yeast species, fusarium and aspergillus in 13 (52%), 5 (20%) and 4 (16%) cases, respectively. Multivariate analysis showed that the probability of B-IFI was lower in patients receiving mold-active antifungals for prophylaxis or empiric therapy than in the pre-emptive setting (OR 0.07) and refractory AML (OR 4.95) and acute GVHD (OR 4.95) were main risk factors. B-IFI patients were treated with more than one antifungal in 37 cases (59,7%). Overall, 35 (56,5%) and 27 (43.5%) cases had a favourable outcome at the end of treatment and at 90 days. Survival at 90 days was 48.3%. Both advanced age and refractory AML are predictors of death in patients with B-IFI (p=0.02 and 0.05) respectively.
Conclusion: This study shows that the pattern of breakthough IFI is moving to yeasts and the higher risks are in the more advanced use of prior antifungals, long lasting neutropenia and acute GVHD.

TABLE 1. Patients' main characteristics

	B-IFI cases	Controls	Total
Number of patients	62	122	184
Age median *y (range)*	55 *(9-78)**	46 *(6-87)*	50 *(6-87)*
Male gender n *(%)*	*(56.5%)*	*(47.5%)*	*(50.5%)*
Diagnosis			
AML	36 *(58.1%)*	73 *(59.8%)*	109 *(59.2%)*
ALL	7 *(11.3%)*	11 *(9.1%)*	18 *(9.8%)*
Lymphoid neoplasm	16 *(25.8%)*	37*(30.3%)*	53 *(28.8%)*
Other	3 *(4.8%)*	1 *(0.8%)*	4 *(2.2%)*
Dis. relapse/refractory	22 *(35.5%)*	35 *(28.7%)*	57 *(31.0%)*
Allogeneic transplant	16 *(25.8%)*	23 *(18.9%)*	39 *(21.2%)*
with acute GVHD	10 *(16.1%)**	8 *(6.6%)*	18 *(9.78%)*

* p<0.05

P778

Evaluation of intervention strategy based on CMV-specific immune responses after allogeneic SCT

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Prophylaxis and pre-emptive treatment during the first 100 days after allogenic stem cell transplantation (allo-SCT) reduced morbidity and mortality caused by CMV. Due to side effects, it is vital to identify patients with high risk to develop CMV disease and need of antiviral therapy after day 100. To steer antiviral therapy in transplanted patients based on monitoring CMV specific immunity after day 100. 80 allo-SCT patients were studied at 4,5.5 and 7 months after SCT.
CMV reactivation was measured by RT-PCR and CD4 and CD8 T-cell responses were analyzed by stimulation of peripheral blood lymphocytes with CMV antigen AD-169 and pp 65 (HCMV Antigenemia) respectively. The interacellular INF-delta production was analyzed by flow cytometry and Elispot.
Antiviral therapy was deferred in patients with documented CMV-specific immunity and no symptoms of CMV disease or severe GVHD.
27 episodes of CMV reactivation later than 3 months after SCT were available for evaluation. The strategy was correctly applied in 18/27 episodes.
Therapy was deferred in 4/27,none of these patients developed CMV disease and was correctly given according to the strategy in 11/27 episodes, one of these patients developed CMV disease. Two patients received antiviral therapy despite having T-cell specific immunity.
A suggestive association was found between late CMV reactivation after SCT and weak CD8 T-Cell immunity at 3 months (p=.04).There was no difference in CD4 immunity.
Monitoring of SCT patients immunity during CMV reactivation may allow a more targeted use of antiviral therapy.

P779

Clinical factors associated with hepatitis B virus seroconversion in HBsAg-positive recipients undergoing allogeneic aematopoietic stem cell transplantation with HBsAg-negative donors

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Objectives: In clinical practice, it is often observed that some HBsAg positive recipients undergoing allo-HSCT with HBsAg negative donors develop seroconversion of hepatitis B virus (HBV) while others still keep positive for HBsAg. What lead to

the two opposite results in clinic is elusive. In the current study, we performed a retrospective analysis of HBV seroconversion in this special population and attempted to find out some clinical factors associated with this seroconversion.
Methods: Between 2005 and September 2010, 26 HBsAg positive patients underwent allo-HSCT with HBsAg negative donors were enrolled. HBV seroconversion was defined as positive HBsAg disappeared within 6 months post allo-HSCT. Patient information was collected from the BMT database. Anti-HBV therapy consisted of lamivudine or entecavir for HBsAg positive recipients before HSCT while marrow harvest and HSCT were performed until recipient's serum HBV-DNA became undetectable. HBV-DNA was isolated from serum with the QIAmpt blood kit and quantitatively measured using a kinetic fluorescence detection system. All of the patients were followed up in outpatient department weekly and HBV serology were detected once every month. Patients who were negative for HBsAg following allo-HSCT were regarded as group 1 and those still with positive HBsAg were regarded as group 2. Donor type, HLA disparity, incidence of aGVHD and cGVHD, HBsAb level in donor, primary disease, conditioning regimen containing ATG and sex disparity were compared between the two groups by SAS 8.2.
Results: 15 of 26 patients converted to negative HBsAg (group 1) and the other 11 patients still kept positive for HBsAg (group 2). In group 1, 5 received HSCs from related donors (33.3%) compared with 8 (72.7%) in group 2 which were statistically different (p=0.0476). In total 5 (33.3%) and 5 (33.3%) of 15 patients in group 1 developed aGVHD and cGVHD versus 6 (54.5%) and 4 (36.4%) of 11 patients in group 2 respectively (P>0.05). Also multivariate analysis showed that primary disease type, donor and recipient's sex, HBsAb level in donors and conditioning regimen containing ATG were not associated with the seroconversion statistically.
Conclusion: Our data firstly suggests that unrelated donor type is the only clinical factor that makes the HBsAg positive recipients more proned to HBV seroconversion post allo-HSCT with HBsAg negative donors. Large scale of clinical trials should be carried out in the future.

P780
Detection and quantification of HAdVs in patients undergoing allogeneic HSCT using qPCR
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Objectives: Adenovirus infections are associated with significant rates of morbidity and mortality among patients after haematopoietic stem cell transplantation (HSCT). Only few transplantation units use molecular virological methods, such as polymerase chain reaction (PCR), for surveillance for adenovirus infection, and treatment strategies have never been evaluated in multicenter clinical trials.
Materials and methods: We retrospectively tested serum samples obtained from a cohort of adults who had undergone allogeneic HSCT. A total of 451 samples collected from 57 patients

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Table 1. Virological and clinical characteristics of the study group during the early post-transplantation period

Median days post HSCT to detection of adenoviruses	49
Range of days post HSCT to detection of adenoviruses	19 - 70
Median of HAdV positive sera samples	4
Range of HAdV positive sera samples	3 - 7
Median of HAdV DNA quantity (copies/ml)	$4.32 \cdot 10^{3}$
Range of HAdV DNA quantity (copies/ml)	$1{,}12 \cdot 10^{2}$ - $2.86 \cdot 10^{4}$
Signs and symptoms of infection	**Number of patients**
Fever	29
Cough	9
Pneumonitis	7
Diarrhoea	17
Pulmonary infiltrates in chest X-ray	9
Acute GvHD	6
Other viruses found during HAdV viraemia	
Human herpesvirus-1, -2 (HHV-1, -2)	1
Human herpesvirus-3 (HHV-3)	-
Human herpesvirus-4 (HHV-4)	-
Human herpesvirus-5 (HHV-5)	5
Human herpesvirus-6 (HHV-6)	-
Human herpesvirus-7 (HHV-7)	6

between January 2007 and December 2009 were tested for adenovirus infection by quantitative real-time PCR. Also sequencing of a part of HAdV hexon-proteine encoding gene was performed for determination of adenovirus subgroup.
Results: Evidence of adenoviral DNA was found in 47 sera samples (10.4%), taken from 11 individuals (19.3%). The values of 600–12500 copies/ml predominated, representing a low-to-medium level. Viraemia was observed between day 19 and 70 after transplantation.
As the result of sequencing part of adenoviral gene encoding hexon protein a subgroup B1, subgroup B2 and group C was determined in 46%, 36% and 18% of the patients, respectively.
Conclusions: HAdV infections are wide spread within group of patients undergoing allogeneic HSCT. Molecular laboratory tests for adenoviral DNA may assist clinicians in decision whether antiviral therapy should be administered to patients who develop adenovirus viraemia.

P781

A survey of practice relating to the prevention and treatment of invasive fungal infections in UK transplant centres

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Invasive fungal infections (IFIs) are a major cause of morbidity and mortality in patients with haematological malignancies. Although guidelines exist for the management of IFIs in this patient group, practice has been shown to vary widely between European haematology centres. The aim of the current study was to investigate whether similar variability exists in the United Kingdom or whether recently published guidelines have helped to standardise practice.
A short questionnaire was sent electronically to all members of the UKBMT pharmacists' group. Questions focused on prophylaxis and treatment of fungal infection in three specific clinical areas – AML induction, autologous stem cell transplantation (SCT) and allogeneic SCT.
Responses were received from 29 centres. All centres treated autologous SCT and AML patients and 27 treated allogeneic SCT patients. Itraconazole and fluconazole were the most widely used prophylactic agents although only 72% of centres gave prophylaxis to all three patients groups. Variability existed between patient groups with fluconazole (dose range 50-400 mg/day) being most widely used in patients undergoing Autologous SCT (42% of centres) but itraconazole being preferred in the settings of both allogeneic SCT (56%) and AML induction (60%). Four centres (14%) gave posaconazole prophylaxis during AML induction. Empirical therapy was recommended by 22 centres (76%). Ambisome® (dose range 1-3 mg/kg/day) was the most widely used empirical antifungal agent and it was also the first line drug for the management of invasive aspergillosis in 50% of antifungal policies. Voriconazole and caspofungin were recommended as the first line antifungal agent in 23% and 20% of policies respectively. Other first line agents were Abelcet® (7%) and posaconazole (3%). No centres used conventional amphotericin B. Caspofungin was the most popular second line agent, being recommended in 40% of policies, followed by voriconazole (30%) and Ambisome (25%). Twenty centres (69%) would consider giving dual therapy, with combinations of lipid amphotericin plus caspofungin (37%) and lipid amphotericin plus voriconazole (37%) being most commonly recommended.
Similar to an earlier Europe-wide survey, this study showed significant variations in practice between UK haematology centres with respect to both preventing and treating invasive fungal infections. Recent guidelines in this area do not appear to have resulted in a standardisation of practice.

P782

Enhanced immune response after a second dose of an AS03-adjuvanted H1N1 influenza. A vaccine in patients after haematopoietic stem cell transplantation

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Objectives: To determine the rate of seroconversion after one or two doses of a novel split virion, inactivated, AS03-adjuvanted pandemic H1N1 influenza vaccine (A/California/7/2009) in patients with hematologic malignancies after hematopoietic stem cell transplantation (HSCT) (ClinicalTrials.gov Identifier: NCT01017172).
Methods: Diagnostic study of adult patients with hematologic malignancies after HSCT scheduled for H1N1 influenza A vaccination. Blood samples were taken before and 21 days after a first dose and 21 days after a second dose of the vaccine. Antibody (AB) titers were determined by haemagglutination inhibition assay. Seroconversion was defined by either an AB titer of ≤1:10 before and of ≥1:40 after or ≥1:10 before and ≥4 fold increase in AB titer 21 days after vaccination.
Results: Seventeen patients (14 allogeneic, 3 autologous HSCT) received one dose and 11 of these patients two doses of the vaccine. The rate of seroconversion was 41.1% (95% confidence interval (CI) 59.6–75.9) after the first and 81.8% (95% CI 85.9–95.9) after the second dose. Patients who failed to seroconvert after one dose of the vaccine were more likely to receive any immunosuppressive agent (p=0.003), but time elapsed after or type of HSCT, age, sex or chronic graft versus host disease were not different when compared to patients with seroconversion.
Conclusion: In patients with malignancies after HSCT the rate of seroconversion after a first dose of an adjuvanted H1N1 influenza A vaccine was poor, but increased after a second dose.

P783

Incidence of viral infections in adult recipients of double umbilical cord blood transplantation

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Viral infection is a major cause of morbidity in umbilical cord blood transplantation (UCBT). We recorded cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus-6 (HHV-6) and BK virus (BKV) infection in adult recipients of double UCBT. 26 patients received double UCBT for hematologic malignancy, at a median age of 38 (range, 16-60) years, with myeloablative (n=18) or reduced-intensity (n=8) conditioning. Antithymocyte globulin was used in 1 case. The majority of UCB units (43/52) were 4/6 matched to recipient at HLA-A, -B, and -DRB1, whereas by allele typing 29 (55.8%) were ≥4/6 and 23 (44.2%) <4/6 matched. Patients were monitored for CMV, EBV and HHV-6 reactivation with real-time quantitative PCR (RQ-PCR) in blood weekly until day 180 or longer if on continued immunosuppression. Urine samples were examined for BKV by RQ-PCR in the evidence of hemorrhagic cystitis (HC). The cumulative incidence (CI) of CMV infection was 34.6%, and occurred at a median time of 61 days (range, 25-116). Among 9 cases of CMV infection, 6 had recurrent viraemia after initial treatment. One case of CMV enteritis was documented. Pre-transplant CMV serostatus predicted for higher CMV infection incidence although the difference did not reach significance (CI 42.11% vs. 14.29% in seropositive and seronegative recipients, respectively; HR=3.72; p=0.19). Higher CD34+ cell dose (>$0.8x10^5$/kg) and high-resolution HLA match (≥4/6 allele) of the engrafting unit seemed protective from CMV reactivation on univariate analysis (HR=0.23 and 0.29; p=0.07 and 0.1, respectively). CMV infection was marginally a risk factor for

poorer overall survival among day +100 survivors (p=0.07). The CI of HHV-6 reactivation was 61.5%, and it occurred earlier than CMV infection, at a median of 32.5 (range, 18-139) days. Twelve of 16 patients with HHV-6 infection were treated with foscarnet due to myelosuppression (n=11) or encephalitis (n=1). Only 2 of these had concurrent CMV infection. The CI of BKV detection in urine in association with HC reached 40.7%. Median time to onset of BKV-HC was 27.5 (range, 12-80) days with a median viral load of $4.5x10^8$ (range, 0.27-$970x10^8$) copies/ml. Cidofovir was administered in 5 of 11 patients with BKV-HC, and cystoscopy was required in 3. There were 3 cases of EBV reactivation (CI=12.16%), with lethal EBV-related lymphoproliferation in 1 patient. In conclusion, in double UCBT surveillance and prompt treatment of viral infections besides CMV is mandatory.

P784

Value of chest X-ray in the diagnosis of lung infections during neutropenic phase of reduced-intensity allogeneic transplantation

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Background: Lung infection is a frequent complication of neutropenic phase after allogeneic transplantation (alloSCT). The first exam usually performed to detect lung infections is chest X-ray (CXR), but its diagnostic value in this setting is a matter of debate. The gold standard imaging tool to detect lung infections is high resolution CT scan (HR-CT).
Objectives: The aim of this study was to understand the value of CXR to detect lung infections during the neutropenic phase in patients receiving alloSCT following reduced intensity conditioning (RIC).
Methods. We reviewed the CXR and HR-CT scans performed from day 0 to day +30 of 236 patients who received RIC alloSCT at our center between 2002 and 2009. Median age of patients was 49 years (range, 15-68), diagnoses were: leukemia (53 patients), lymphoma (119), multiple myeloma (49), and solid tumors (15). All patients received peripheral blood stem cells from HLA identical siblings (110), matched unrelated (83) or haploidentical donors (43). CXR was performed in 171 patients at a median of 13 days (range, 0-27) after alloSCT. Forty-five patients had respiratory symptoms or persistent fever of unknown origin (FUO); these patients were evaluated with both CXR and HR-CT scan, which was considered the reference standard to assess sensibility, specificity, positive predictive value (PPV) and negative predictive value (NPV) of CXR.
Results: Of 45 patients evaluated with CXR and HR-CT, 19 had evidence of lung infection at CXR. The sites of infection were: low inferior right lobe (9), low inferior left lobe (7), median right lobe (3), left upper lobe (1); 6 patients had pleural effusion, 3 had interstitial infiltrates. Of 19 patients with positive CXR, 18 had diagnosis of lung infection confirmed by HR-CT scan, whereas 1 patient had negative HR-CT scan and did not develop clinical sign of pneumonia. Lung infection was diagnosed by HR-CT scan in 5 of 26 patients with a negative CXR. Sensibility of CXR was 78% (IC95% 61%-95%), specificity was 95% (IC95% 86%-100%), PPV was 95% (IC95% 86%-100%), NPV was 81% (IC95% 66%-96%).
Conclusions: CXR has high specificity and PPV to assess lung infections during neutropenic phase in RIC alloSCT patients. Confirmatory HR-CT could be avoided in patients with positive CXR, obviating radiation exposure and the costs of HR-CT. Instead, the low sensibility and NPV of CXR suggest that patients with negative CXR and with respiratory symptoms or FUO should promptly perform HR-CT scan.

P785

Pre-emptive treatment for CMV reactivation after allogeneic stem cell transplantation: a single-centre experience in 223 patients

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Background: Cytomegalovirus (CMV) reactivation remains an important issue after allogeneic stem cell transplantation (allo-SCT).
Aims: We report the retrospective analysis of CMV preemptive therapy in patients (pts) experiencing CMV reactivation after allo-SCT in the last 6 years.
Materials and methods: Between November 2004 and October 2010, 223 pts (median age 49; 142 males) received transplant for high risk haematological malignancies; 7 pts received a second transplant; 54 from HLA identical sibling, 57 from unrelated volunteer, 112 from family haploidentical donor (20 with and 92 without ex-vivo T-cell depletion), 7 cord blood. Pts were evaluated for CMV quantitative pp65 antigenemia and/or quantitative DNA PCR twice weekly within the first 3 months after allo-SCT. Host/donor serostatus was: -/-16, +/+124, -/+40, ±50. Response to treatment was defined as negativization of antigenemia or DNA for at least 2 consecutive determinations.
Results: We observed CMV reactivation in 125/223 pts (56%), median time of onset 36 (0-91) days post Allo-SCT, median number of CMV Ag 3 (1-35) nuclei and PCR 1903 (114-66000) cp/mL. Baseline hematological recovery was similar in all groups. 58 pts received oral valganciclovir (VCGV) as first line preemptive therapy; median time of treatment was 17 (2-44) days. The reactivation was solved in 44 pts (75.9%), 17 required drug crossover. Hematological toxicities were detected in 33 pts (56.9%). Severe infectious adverse events with hospitalization (SAE) were observed in 7 pts with grade III-IV neutropenia. No further reactivation was reported in 24 pts. Intravenous preemptive therapy was administered in 26 pts: 17 ganciclovir (GCV), 8 foscarnet (FCV); median time of treatment was 12.5 days (range 1-28). Events completely solved in 23 pts (88.5%), 10 required drug crossover. Hematological toxicities were detected in 7 pts (26.9%). SAE was reported in a patient with grade IV neutropenia. No further reactivation was reported in 11 pts.
Conclusions: VGCV, GCV and FCV treatments are effective (p 0.2462) as first line therapy early after allo-SCT. VGCV seems to be significantly associated with more hematological toxicities (p 0.0174) and related SAE as compared to GCV and FCV; these data suggest the safer use of intravenous therapy in the first 3 months after allo-SCT.

	VGCV	GCV/FCV
Pts	58	26
Efficacy	44 pts	23 pts
SAE	7 pts	1 pts
Haematological Toxicities		
Overall	33 pts	7 pts
Neutropenia grade IV	18	2
Neutropenia grade III	14	3
Anemia grade III	7	2
Thrombocytopenia grade IV	1	1
Thrombocytopenia grade III	15	2

P786

H1N1 vaccination post-allogeneic haematopoietic stem cell transplantation and the risk of GvHD

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Background: In 2009 the H1N1 influenza outbreak prompted the recommendation to vaccinate all patients (pts) receiving allogeneic hematopoietic stem cell transplantation (HSCT).

Although the benefits towards influenza incidence and severity were anticipated, the risk of precipitating graft-versus-host disease (GvHD) has been taken into account, due to the capability of H1N1 vaccination to stimulate the innate immune system.
Aim: To evaluate the efficacy and safety of H1N1 vaccination in HSCT patients.
Patients and methods: Between November 2009 and March 2010, we vaccinated 29 pts after HSCT: 13 HLA-identical sibling (Sib), 9 matched unrelated, 5 haploidentical and 2 cord blood (CB) donor; median time from transplant to vaccination was +560 days (range 100-2090); according to institutional protocols GvHD prophylaxis has been Cyclosporine (CSA) in 23 pts, Rapamycin and Mycophenolate in 6; 22 pts had a history of previous GvHD. Ten pts had active GvHD at time of vaccination and were under immunosuppressive therapy (IS), 6 of them in tapering. In parallel, we vaccinated as controls 21 pts suffering from hematological malignancies: 13 treated with chemotherapy (CT), 8 with autologous transplant (ASCT). All patients received Focetria®, a monovalent H1N1 vaccine with MF59® adjuvant. Pts after CT received one-dose of vaccine, while a second dose was administered 1 month later in pts having ASCT or allo-SCT (from day + 100).
Results: The vaccination was well tolerated in all pts. There was only one case of H1N1 infection, in a patient post ASCT. Lymphocytosis was observed in 5 pts (HSCT 3, controls 2), transient in 4, persistent in one at last follow up. Three HSCT pts (2 Sib, 1 CB) experienced exacerbation of GvHD and one sib patient had a new onset GvHD, with a median time after vaccination of 24 days (11-29), without any correlation with lymphocytosis. Three pts were tapering IS and one had recently discontinued (2/4 CSA, 2/4 steroid). All 4 cases were chronic GvHD, 2 low and 2 moderate grade (3/4 involvement of skin, 2/4 eyes, 2/4 liver). All GvHD events were completely solved after appropriate IS treatment (2 pts increased CSA, 1 switched from CSA to Rapamycin, 1 restarted steroid).
Conclusions: Influenza vaccination in HSCT pts is safe and well-tolerated. However these data suggest that tapering of IS should be avoided within the first few months.

P787
Are new tools able to improve microbial documentation of febrile neutropenia in stem cell transplant and acute leukaemia patients?
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Febrile neutropenia (FN) episodes are documented by routine blood cultures (BC) only in 30% of cases. Whether this low rate of documentation is due to low bacterial inoculum or non-infectious causes of fever is currently unknown. We hypothesized that a combination of early blood incubation, and use of the most efficient and rapid techniques for positivity detection could improve the diagnostic yield of BC in FN. We prospectively assessed a new process for microbial documentation of FN, combining (i) 1 x 40 ml-BC with 4 bottles; (ii) immediate incubation in an automate implemented in the hematology ward (BacT/Alert3D®); (iii) microbial DNAemia detection using the LightCycler Septifast® test and (iv) rapid identification and susceptibility testing methods (Vitek2® (Biomérieux) and Genotype® (Hain Lifescience)) applied directly on BC when positive. This was compared to standard BC procedure. All adult patients hospitalized in the hematology ward from Feb 2008 to Mar 2009 were eligible at their first episode of FN if they were neutropenic (PMN<0.5 x10^9/L), febrile (temperature ≥ 38°3 or twice ≥ 38° within 8 h), and had a central venous catheter. One hundred and 20 consecutive episodes of FN, including 34 episodes in stem cell transplant recipients, were consecutively included. The BC positivity rate was 28.3% (34 episodes) with the routine procedure vs 30% (36 episodes) with the new process (McNemar's chi2=0.67, p=0.41). Septifast® test was positive in 9 episodes (7.5%) that were also positive by routine BC. Time to positivity was significantly shorter for the BC incubated in the ward (median rank: 12h31 [7h55-25h37]) than for routine BC (median rank: 13h01 [9h31-19h27] (p=0.004). Time to results of identification and susceptibility testing using rapid biochemical and molecular tests produced results within 24 hours (mean 19h46 [13h30-40h09]).
Conclusion: Immediate BC incubation combined with DNA detection directly on blood without culture (Septifast test) does not improve the rate of BC positivity in FN episodes, when compared to routine process. Our main result is to have shortened the time, first, for BC positivity when BC bottles were incubated in the ward and second, for identification and susceptibility testing by using GenoType and Vitek2 tests directly on positive BCs. Whether this gain translates into a clinical benefit, either in early adaptation of the first-line antibacterials, or in FN survival, needs further studies.

P788
The phantom of the opera: when prophylaxis acts
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Introduction: We undertook a retrospective analysis of the episodes of sepsis occurred during the first 30 days after allogenic hematopoietic stem cell transplantation (HSCT) in 124 consecutive children transplanted in our hospital from 1995 to 2009.
Methods: The population was divided in 3 groups according to the year of HSCT (group 1: 1995-99; group 2: 2000-04; group 3: 2005-2009). For each group, demographic characteristics, underling disease, type of donor, cells source, time of engraftment and incidence of mucositis (classified following WHO criteria and defined severe if grade 3 or 4) were considered. All children received myeloablative conditioning regimen. As prophylaxis for infections, they were cared for in single rooms with positive pressure and high-efficiency particulate air filtration. Moreover, fluoroquinolones (from day -1), fluconazole (from day + 3), pentaglobin (from 2007) as prophylaxis for sepsis (i.e. if low fever or other sign of infections during aplasia) and immunoglobulin (after engraftment) were administered. In case of fever (pyrexia > 38 ° C) or other signs or symptoms of infection, prophylactic antibiotics patients were treated with broad-spectrum antibiotics. The incidence of clinical sepsis (bacteremia, invasive mycosis or unknown origin) during the first 30 days after transplant was measured.
Results: Group 1, 2 and 3, were composed by 27, 42 and 55 patients respectively. The groups did not significantly differ for demographic characteristics, underling disease, type of donor, cells source and time of engraftment. We found a decreased incidence of severe mucositis (p=0.00) over the time; it was 18/27 (67%) in group 1; 12/42 (29%) in group 2; 5/55 (9%) in group 3. The incidence of clinical sepsis decreased too (p=0.00). In regard to the number of episodes of sepsis, in group 1 there were 15 (55,6%) episodes (6 bacteremia; 3 mycosis; 4 unknown origin; 2 polymicrobic); in group 2 there were 14 (33,3%) episodes (8 bacteremia; 3 mycosis; 3 unknown origin); in group 3 there were 9 (16,3%) episodes (6 bacteremia, 1 mycosis; 2 polymicrobic). There was a strong association between mucositis and sepsis (p=0,003).
Conclusions: In our series the incidence of sepsis diminished consistently in recent years. The reduction of mucositis, associated with more accurate methods of diagnosis and use of antibiotics, might be an important cause of this phenomenon.

P789
Is antifungal prophylaxis useful in stem cell transplantation during hospitalization?
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Introduction: To date anti-fungal systemic prophylaxis is recommended in autologous stem cell transplant (SCT) recipient

subset with prolonged neutropenia and mucosal damage and in allogeneic mieloablative transplant setting. Limited data support the efficacy of antifungal prophylaxis applied to neutropenic and/or immunocompromised patients.
Methods: This is a retrospective study of 1007 SCTs performed in Bone Marrow Unit of Reggio Calabria Hospital between 1992 and 2009 including 809 consecutive patients. We performed environmental monitoring of air, water, surfaces in room with HEPA filter for better infectious risk management.
Results: The main characteristics of the patients are reported in Table 1. Systemic prophylaxis was used according to the guidelines (Table 2). Our therapy scheme was based on administration of fluconazole in the nineties years, followed by itraconazole. During 2004 year, these treatments were abolished or substituted with prophylaxis therapy based on non-adsorbable molecules in transplants with standard infection risk. Secondary prophylaxis was prescribed for high risk patients with fungal infection history, suggestive iconography, positive fungal biomarkers. In allogeneic SCT cohort only 3 probable aspergillosis infections and 5 proven fungal infections (1 fusarium, 1 mucor and 3 aspergillosis) were diagnosed. (2 haplotipical, 1 singenic, 1 sibiling, 1 MUD and 2 mismatched), resulting in death in all cases. The infection rate in allogenic SCT setting was 3.6% with an incidence rate of 1.1 infection per 10000 transplants/year, while no fungal infection was documented in autologous SCT setting. These results are significantly lower than published reports. Finally, during the 17 years our transplant unit has never been colonized by moulds.
Conclusions: Based on our experience, we believe that systemic antifungal prophylaxis should not be performed in autologous SCT patients. except in selected cases. In allogenic SCT the fungal prophylaxis should be tailored to the treatment adopted for the patient (bone marrow suppression, reduced intensity, conditioning) and it has not to be administered in absence of risk factors according to guidelines. The identification of high risk patients is useful to select for systemic antifungal or secondary prophylaxis reducing over-treatment, incidence of resistant strains and costs.

Table 1 - Characteristics of patients underwent to SCT

Number of patients	809
Sex (M/F)	404/405
Number of transplants Single transplant > 1 transplant	1007 805 (79,9%) 202 (21,1%)
Transplants type Autologous Allogeneic	787 (78,1%) 220 (29,9%)
Disease: Acute Leukemia Chronic Mielod Leukemia Lymphoma Myeloma Solid Tumor Others	210 (20,8%) 34 (3,4%) 265 (26,2%) 239 (23,7%) 205 (20,4%) 55 (5,5%)

Table 2 – Fungal type, antifungal strategies and antifungal drugs employed during 1992-2009

Antifungal prophylaxis strategies: Secondary prophylaxis Systemic prophylaxis Non-absorbable prophylaxis No prophylaxis	Autologous 5 (0,6%) 560 (71,1%) 189 (22,9%) 33 (4,4%)	Allogeneic 15 (6,8%) 158 (71,8%) 41 (18,6%) 6 (2,6%)
Antifungal drugs: Fluconazole Itraconazole Posaconazole Voriconazole Caspofungin Amphotericin liposomal Amphotericin suspension Oral Nystatin	319 (42,3%) 235 (31,6%) 4 (0,6%) 2 (0,05%) 3 (0.4%) 2 (0.05%) 181 (24%) 8 (1,1%)	67 (30,4%) 91 (41,4%) 0 2 (0,9%) 2 (0,9%) 13 (6%) 43 (19,5%) 2 (0,9)
Fungal infection type and prophylaxis Aspergillosis (3 Amphotericin liposomal - secondary, 2 Itraconazole- systemic) Mucor (Fluconazole- systemic) Fusarium (Itraconazole- systemic)	0 0 0	5 1 1
Fungal infection Rate	0/787	7/220 (3,1%)

P790
Quantification of CMV DNA in plasma by real-time PCR for monitoring allogeneic stem cell transplant recipients
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The objective was to evaluate rt PCR QIACMV Abbott Diagnostics in plasma samples for CMV monitoring for preemptive treatment of allo-SCT patients.
Methods: We studied 521 plasma samples obtained in 76 CMV infection episodes from 47 allo SCT patients between 2005 and 2008. All patients were monitored post-SCT with antigenemia pp65 CINApool®, Argene (AG) and quantitative PCR, COBAS® Amplicor® CMV after automatic extraction COBAS® Ampliprep® TNAI kit, Roche (CobasPCR). A positive sample was defined by AG>or=2/4x10^5 PMNs and/or CobasPCR>or=600 copies/mL, 41 out of 47 received one or more course of preemptive therapy upon positive AG and/or CobasPCR results and 6 (12.7%) developed CMV end-organ disease (4 colitis and 2 neumonitis). An episode was defined as the period between the first positive sample by AG and/or CobasPCR, until the first negative sample by both techniques. All samples were retrospectively tested using rt-PCR after automatic DNA extraction with m2000sp. Rt-PCR positive results were defined by >or= 52 copies/mL.
Results: Plasma samples were positive in 29.2%, 34% and 40.7% by AG, Cobas PCR and rt-PCR, respectively. Thirty-nine samples (7.5%) were only positive by rt-PCR, of these 9 were first and 15 last samples in episodes. Concordance between PCR assays was 83.7% (k=0.63), Pearson 0.98 (p<0.001) and rt-PCR vs AG& CobasPCR was 80.6% (k=0.66). ROC curve analysis was applied using the existing treatment criteria based on AG and CobasPCR for triggering CMV preemptive therapy and a viral load of >173 copies/ml by rt-PCR was established as the optimal value (S 65%, E 73% AUC 0.728).
Episodes were detected in 71 (93%), 58(76%) and 67(88%) by AG, CobasPCR and rt-PCR, respectively. Nine episodes (11.8%) were only detected by AG (all PCRs negative) and 5 (6.6%) only by PCR assays. For episode detection the first positive technique was AG 56 (74%), rt-PCR 51 (67%) and Cobas PCR 37 (48%); AG was the first positive technique in 26% episodes (median=1 day) and rt-PCR in 10% episodes (median=10 days).Bland Altman Analyses CobasPCR vs rt-PCR difference is 0.76 log.
Summary: Rt PCR QIACMV detects earlier and more episodes than the COBAS ®AMPLICOR PCR CMV. Rt-PCRcuantitative results were lower than end-point PCR (0.76 log). The threshold of 173 copies/mL is the optimal rt-PCR CMV-DNA value for triggering preemptive treatment in allo-SCT. Results from this study suggest that this rt-PCR assay is a useful tool for the clinical management of CMV in allo SCT patients.

P791
Voriconazole prophylaxis in 120 high-risk patients undergoing allogeneic haematopoietic stem cell transplantation
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Background: Invasive fungal infections (IFIs) are emerging serious complications with high mortality in patients (pts) undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). Since 2008 we used voriconazole as antifungal prophylaxis in the allo-HSCT setting, due to pts' high risk condition, donor source, local epidemiology.
Aims: The aim of this study is to evaluate incidence and risk factors for IFIs in high-risk hematological pts receiving allo-HSCT.

Materials and methods: In our Institution we analized 120 consecutive pts with high-risk hematological malignancies who underwent allo-HSCT from January 2008 to May 2010 (9% Matched Related Donor, 30% Matched Unrelated Donor, 47% Haploidentical HSCT, 7% T-depleted Haploidentical HSCT, 7% Cord Blood). Antifungal prophylaxis with voriconazole was primary in 82 pts (68%) and secondary in 38 pts (32%). Disease status at HSCT was intermediate- advanced for 69% of the pts (n 83); Sorror Comorbidity Index (CI) score was > 2 in 36% of the pts (n 43); the number of previous chemotherapy lines was >2 in 36% of the pts (n 43).
Results: Voriconazole drug toxicity was observed in 7% of the pts (n 18): hepatic (n 15), neurological (n 1) and renal (n 2) side effects were observed and cleared up with drug withdrawl. The breakthrough IFIs incidence was overall 32% (n 39): 11% proven (n 13), 2% probable (n 3), 19% possible (n 17) according to EORTC 2008 criteria. We observed IFIs complete resolution in 23/39 pts; first line therapy alone was effective in 15/39 pts. The main site of infection was lower respiratory tract (74%, n 29). The IFIs incidence was 18% within day +30 post HSCT (n 22), 7% from day +30 to day +100 (n 8), 7% after day +100 (n 9). Univariate analysis highlights no statistical significant association (p>0.05) between IFIs and following risk factors: number of previous chemotherapy lines, disease status at HSCT, Sorror CI score, conditioning with anti-thymocyte globulin. The attributable mortality rate for IFI was 3% (n 1).
Conclusions: Voriconazole prophylaxis is a safe and effective treatment in high risk hematological patients undergoing allo-HSCT. In our experience cumulative incidence of proven and probable IFIs was comparable with literature data, with a low infections attributable mortality rate despite unfavorable conditions.

P792

Incidence of infectious complications after HLA-haplo-identical haematopoetic stem cell transplantation using high-dose cyclophosphamide posttransplantation

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Infectious complications are a significant cause of morbidity and mortality after hematopoetic stem cell transplantation (sct). Recipients of haplodentical transplants may be more suspectible to infectious complications due to intensive immunosuppression or T cell depletion resulting in reduction of cellular immunity and delayed immune reconstitution after sct.
We evaluate the posttransplantion occurance of viral, bacterial or fungal pathogens causing infectious complications in recipients of unmanipulated haploidentical grafts using cyclophosphamid posttransplantation. Data of 13 consecutively treated patients between 2009 and 2010 were analyzed with respect to demographic data, diagnosis, remission status, conditioning treatment, stem cell source, clinical course and outcome, grade of acute GvHD and microbiological data until day 100 after transplantation.
Infections could be detected in 12/13 pts. Most frequently observed pathogens were staphylococcus species, pseudomonas aeruginosa, cytomegalievirus (CMV), human herpesvirus 6 (HHV6) and Candida spp. Fever of unknown origin in neuropenia occured in 7 pts. Few probable invasive fungal infections were diagnosed (2/13 pts). In 4/13 pts pulmonary infiltrates were observed. The incidence of CMV reactivation was 50 % for pts at risk (patient and or donor CMV seropositive), no pt developed CMV disease. Only one Epstein Barr virus (EBV) reactivation and no posttransplant lymphoproliferative disease (PTLD) occured, compared to an incidence of EBV reactivation and PTLD of 50% and 12% respectively for 57 patients after haploidentical sct using CD 6 depletion as reported by our group previously.
Incidence of infectious complications after haploidentical sct using high dose cyclophosphamide posttransplantation is moderate with a low incidence of EBV reactivation and no incidence of PTLD compared to a haploidentical transplantation setting using in vitro T cell depletion.

P793

Do we need today antibacterial and antifungal prophylaxis in haematopoietic stem cell transplantation during neutropenia period?

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Introduction: Because of the multiple advances in the setting of HSCT and the increasing rates of drug resistant bacteria, current guidelines for primary antibiotic prophylaxis during neutropenia period may be considered.
Objectives: To analyze incidence, characteristics, mortality and risk factors associated with infections during neutropenia period in patients who underwent HSCT (allo or auto) without antimicrobial prophylaxis in our center.
Patients: From January 2007 to December 2009, 190 patients did not receive chemoprophylaxis, except acyclovir, during neutropenic period. When neutropenic fever appeared, empirical antibacterial treatment and antifungal prophylaxis were started.
Median age was 48 years (8-75), 110 patients were male. Hematological diseases were: AML: 47; ALL: 22; MDS: 13; NHL: 38; HD: 21; MM: 29; CLL: 8; Aplastic Anemia: 5; other: 7. Allo-HSCT was done in 121 patients (unrelated 54) and auto-HSCT in 69. Myeloablative regimen (BUCY, CY-TBI, FluBU4, BEAM, BEAC, Melfalan 200) was administered to 148 patients (78%). Bone marrow was the principal stem cell source in allo-HSCT (89%) and peripheral blood in auto-HSCT (100%). Hickman catheter was used in 74% of patients. Seventy eight percent of patients were isolated in a room with air filtration system (HEPA 55% or LAF 23%). Granulocyte recovery (> 500/mm^3) was seen at 14 days (9-25) and only 20% of patients received G-CSF. Parenteral nutrition was given to 42% of patients although only 38% developed moderate-severe mucositis.
Results: Neutropenic fever was seen in 166 patients (88%). Piperacilin-tazobactam associated with fluconazole was the main treatment (75%). Microbiological isolation was found in 102 patients (62%), being blood culture (75%) the principal localization and Staphylococcus Epidermidis (31%) and Escherichia Coli (8%) the main pathogens. Two patients developed catheter related fungemia by Candida Parapsilosis (1%). Severe infection was observed exclusively in fifteen patients (8%) and only 3 of them died (2%). Young patients (p=0,03), ablative conditioning (p=0,04), Hickman catheter (p=0,001) and parenteral nutrition (p=0,001) were detected as risk factors in the univariate analysis.
Conclusion: In our center, the absence of chemoprophylaxis for bacterial and fungal infections during neutropenia period after HSCT has shown a low rate of mortality. Rupture of barrier is the main factor for febrile neutropenia and gram positive bacteriemia the frequently observed.

P794

Bloodstream infections in patients undergoing autologous stem cell transplantation - the rising problem of resistant pathogens

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Bloodstream infections (BSIs) are major cause of morbidity and mortality in patients undergoing autologous peripheral blood stem cell transplantation (auto-PBSCT). Knowledge of local epidemiology is a prerequisite for their optimal

management. The aim of this study was to characterize the epidemiology of BSIs in patients treated with auto-PBSCT in a single institution.
146 consecutive auto-PBSCT performed between January 2008 and December 2009 were evaluated. Follow up was done one year after auto-PBSCT, blood culture dates and isolates were recorded. All patients received oral antimicrobial prophylaxis with ciprofloxacin and fluconazole from the beginning of conditioning therapy until fever, when empirical intravenous antimicrobials were started.
Overall, 59 BSIs were detected at a median of 8 days post auto-PBSCT (range 4 to 358, SD 75), with the majority of BSIs observed in the first month following auto-PBSCT, mostly during the first 2 weeks. (84.7% of all BSIs). In total, 10 different microorganisms were identified as causative agents of BSIs. Gram positive pathogens were the most frequent etiologic agents, responsible for 71.2% of all BSIs. In this group, the most frequently isolated pathogens were Coagulase negative staphylococci which accounted for 55.9% of all BSI and 78.5% of all Gram positive BSIs. Gram negative microorganisms accounted for 28.8% of all BSIs with the single most frequently isolated microorganism Pseudomonas aeruginosa. This pathogen accounted for 13.5% of all BSIs and for 47% of all Gram negative BSIs. Altogether, P. aeruginosa was isolated in 7 patients: 4 of these seven isolates were multidrug resistant (MDR), including resistance to carbapenems and sensitive only to colistin. Anaerobic bacteria were isolated in one patient. There were no fungemias.
In our study BSIs were a frequent complication of auto-PBSCT, with highest incidence during chemotherapy induced mucosal damage and neutropenia. Problem of infections caused by resistant microorganisms rises due to the broad use of antimicrobial prophylaxis. In our study we identified a high number of BSIs caused by Pseudomonas aeruginosa with more than 57% of these isolates showing multiple antibiotic resistance. The treatment of BSI-s caused by Pseudomonas aeruginosa remains a challenge because of the possibility that they may not always be covered with empirical therapy.

P795

Is it possible to replace pre-emptive low dose valgancyclovir treatment instead of standard gancyclovir treatment?

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Introduction: Cytomegalovirus (CMV) infection is a major infectious complication after allogeneic hematopoietic stem cell transplantation (ASCT). In the present study we aimed to evaluated the efficacy and safety of low dose valganciclovir (VGV) for preemptive therapy of CMV infection after ASCT.
Patients and methods: We retrospectively evaluated the data of 177 patients who underwent ASCT between November 2007 and July 2010 at Erciyes University bone marrow transplantation center. The patients who were clinically asymptomatic and given VGV or gancyclovir (GCV) due to CMV seropositivity included into the study. Any number of CMV DNA copies over 235 copies/10^6 peripheral blood leukocyte was classifed as a positive CMV-PCR. CMV infection was defined as 1 or 2 consecutive pp65 antigenemia assays and/or 1 or 2 consecutive positive PCR assays within 1 week. In the preemptive treatment of CMV infection VGV was used 900 mg once a day for 21 days or GCV was as a dose of 5 mg/kg once a day for 21 days. Treatment success was defined as negative values of two consecutive CMV PCR results and/or the absence of CMV antigenemia.
Results: 96 (54%) patients showed CMV reactivation. 44 activation was detected in the 41 patients who were treated with VGV and 23 activation was detected in the group who were treated with GCV. All the patients were given GVHD prophylaxis and all were under immunosuppressive therapy. When the patients were evaluated it was seen that in the VGV group 3 patients (6.8%) had positive CMV results on the fifth week but there was no continuous positive CMV result in GCV group (p>0.2). When the both groups were compared according to the drug toxicity grade I-II neutropenia and grade III-IV thrombocytopenia was lower in the VGV group (p= 0.010 vs p= 0.017 respectively).
Conclusion: Lower-dose oral valganciclovir (900 mg/day) has a comparable efficacy to the proposed standard dose of GCV. Preemptive strategy of oral low dose VGV appears to be safe and effective for the prevention of CMV disease in ASCT patients. In conclusion, large prospective randomized studies are required to better define the dosage, duration and resistance of preemptive therapy with VGV after ASCT.

P796

Comparison of itraconacole, voriconazole and posaconazole as oral antifungal prophylaxis in paediatric patients following allogeneic stem cell transplantation

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Background: Pediatric patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) are at high risk of acquiring fungal infections. Oral antifungal prophylaxis with extended spectra azoles, e.g. voriconazole or itraconazole, is preferentially used in pediatric patients after allogeneic HSCT, while only few studies have been published. We also administered posaconazole based on data from studies in adult patients. Retrospectively, we assessed the safety, feasibility and initial data on efficacy of itraconazole, voriconazole and posaconazole in pediatric patients and adolescents after high dose chemotherapy and HSCT.
Patients and methods: The patient cohorts consisted of children who underwent allogeneic stem cell transplantation for hemato-oncological malignancies and inborn errors of metabolism between January 2004 and November 2010. 30 pediatric patients received posaconazole, 40 pediatric patients received voriconazole and 60 pediatric patients received itraconazole as oral antifungal prophylaxis. The observation period was defined as the time from treatment start of oral prophylaxis until the end of oral antimycotic prophylaxis with a maximum of 200 days after transplant.
Results: No incidence of proven or probable invasive mycosis was observed in all three groups during the observation period according to EORTC definitions (De Pauw et al. 2008). The occurrence of possible fungal infections according to EORTC definitions (Kontoyiannis et al. 2003) in the voriconazole group (37.5%) was significantly different (P < 0.01) from the posaconazole group (10%). Clinical adverse events were observed in five cases in the itraconazole group, in four cases in the voriconazole group and in one case in the posaconazole group. There were no signs of hematotoxicity in all three groups. After start of oral antifungal prophylaxis a significant increase of alanin-aminotransferase (ALT) over the age adjusted upper normal limit was observed in all three groups. A significant increase over the age adjusted limit of γ-GT was only observed in the voriconazole (P<0.01) group. In the itraconazole and voriconazole groups there was a decrease of potassium during antifungal treatment. All changes normalized after discontinuation of prophylaxis.
Conclusion: Taken together, itraconazole, voriconazole and posaconazole showed comparable effectiveness as antifungal prophylaxis in pediatric patients after allogeneic HSCT.

P797

Progressive multifocal leukoencephalopathy in non-Hodgkin's lymphoma transplant patients

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Introduction: Recent studies suggest an increased incidence of JC virus-related progressive multifocal leukoencephalopathy (PML) in non-Hodgkin lymphoma (NHL) patients treated with rituximab. The precise mechanism for this fatal disease remains obscure and its relationship to stem cell transplantation (SCT) is unclear. The current study investigates the incidence, clinical characteristics, risk factors and outcome of NHL patients, diagnosed with PML following SCT.
Methods: Data on 432 consecutive NHL patients, treated with rituximab-containing regimens between Jan 2007 and Nov 2010, were reviewed. NHL type, number of rituximab courses and previous SCT, PML infection, therapy and outcome were recorded.
Results and conclusions: 432 patients (55% with aggressive and 45% with indolent NHL) were reviewed. Median number of rituximab courses was 8 (4-14). 24 patients underwent autologous (n=13) or allogeneic (n=11) SCT. Two patients (0.046%) aged 58 (female) and 50 years (male), both with transformed follicular lymphoma, developed PML. These individuals were heavily pretreated (14 and 10 prior rituximab courses), including autograft (female) and a reduced intensity matched sibling allograft, both performed in complete remission, 8 and 9 months prior to PML detection. Last rituximab dose was administered 1 month prior to transplant. The allografted patient was treated with tacrolimus, steroids and photopheresis for GvHD. PML was diagnosed in the presence of typical MRI imaging and a positive PCR for JC virus (1- in brain biopsy and 1 in CSF). Both patients were severely hypogamaglobulinemic (IgG<200) and lymphopenic (CD4=80 and 25, respectively). The autografted patient was treated with repeated courses of IV immunoglobulin, with no clinical improvement, and died of PML within 3 months. In the allograftd patient, immunosuppressive therapy was discontinued and mefloquine and liposomal cidofovir were administered. Despite an initial 5-fold reduction in viral load, there was no clinical improvement. Following immunosuppressive therapy renewal due to severe GvHD, the patient experienced a rapid deterioration, leading to death, within 2 months since PML detection. Interestingly, there were no PML cases in the non-transplanted NHL patients treated with rituximab, suggesting an additive contribution of profound immunosuppression caused by HDT, to PML development.

P798

Clinical features and outcome of respiratory syncitial virus infection in 26 haematologic disorders patients

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Background: Respiratory syncitial virus (RSV) is a recognized cause of severe respiratory infections in hematologic patients, especially in recipients of hematopoietic cell transplant (HCT). We aim to describe the clinical features and outcome of this infection in our center.
Patients and methods: prospective observacional study among patients followed in our Unit between September, 2009 and April, 2010. Screening was performed for RSV on every patient presenting respiratory symptoms. RSV diagnosis was performed by enzyme immunoassay (EIA) on nasopharyngeal wash.
Results: From 313 respiratory samples taken in this period, RSV was identified in 28 (9%) from 26 patients 61% males, median age 52 years (range 13-74). The underlying disease was: acute leukemia 50% (n=14), non-Hodgkin lymphoma 21% (n=6), multiple myeloma 11% (n=3), Hodgkin lymphoma 7% (n=2), other 11%. From the 28 positive samples, 23 (82%) were diagnosed in the outpatient department and only 2 needed later admission. The most frequent symptoms were: cough (74%), runny nose (67%) and sore throat (41%), followed by fever (33%), dyspnea (18%) and headache (11%). 7% (n=2) of cases had a low neutrophil count (<800/mL) and 14% (n=4) were lymphopenic (< 500/mL). In 4 cases (14%) there was a coinfeccion by another microorganism. 19 patients were HCT recipients (73%): 1 (5%) autologous and 18 (95%) allogeneic HCT; 10 (57%) of them received a reduced intensity regimen. The main source of stem cells was peripheral blood, 76% from related donors. 58% had GVHD at the time of onset and 42% were under immunossuppresor treatment. Median day of onset after HCT was +811 (range 0-2527), with only one case before the +100 day. Most patients had an X-ray performed at onset of symptoms, only on 3 patients significant changes were present, one being an HCT recipient. From the whole group, on 82% patients, antibacterial treatment was started at the onset of symptoms and were closely followed. No patients developed respiratory dysfunction or needed the administration of specific antiviral therapy. There were no deaths attributable to RSV infection.
Conclusions: The morbi-mortality associated with RSV infection on this population was significantly lower than the previously reported. This is probably related to the characteristic of the population and the late median time of onset on HCT patients. The close vigilance and follow up, without the administration of specific antiviral treatment, may be a good procedure in these patients.

P799

HLA mismatch is a strong factor for HHV6 infection post BMT which might become obscured due to treatment for concurrent CMV infection

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HHV6 infection is considered to be a relatively common complication following BMT; however its clinical significance remains unclear. Here we have retrospectively analysed 202 samples from 18 patients who underwent a SCT. 18 patients (11 males, 7 females) with a median age 46 (15-66) at the time of the transplant underwent SCT for leukaemia (n=9), lymphoma (n=5) and others (n=4). The donor was identical sibling in 5 cases, haploidentical in 1, MUD in 11 cases and CB in one case. The source of cells was PBCS (n=16), BM (n=1), CBC (n=1). 17 patients received a RIC transplant, one patient a fully myeloablative. After transplant the HHV-6 load was measured by RT-PCR in 202 samples upon indication. 104 samples were positive. 28 samples had low level HHV6 DNA (<999 c/ml), 39 samples mid-level HHV6 DNA (1000-9999 c/ml) and 37 samples high level HHV6 DNA (>10000 c/ml). 10/18 patients had concurrent CMV infection, rhinovirus (n=1) or increased copies of EBV DNA (n=1). The median time of HHV6 highest positive samples from transplantation was 66 days (14-417). At that time the median lymphocyte count was $0,3x10^9$/L. All patients who transplanted with mismatched grafts developed HHV6 infection. Six patients had at least one low level (n=2) or mid-level (n=4) positive sample for HHV6. They were all followed without treatment.12 patients had at least one HHV6 measurement above 10000 c/ml. 2 patients developed HHV6 encephalitis, 1 patient HHV6 related marrow aplasia and 9 patients lung pathology. One patient had continuously high levels of HHV6 copies and was considered to have chromosomal integration. 7 patients had also concurrent CMV infection and were treated with ganciclovir or foscarnet. From the remaining 5 patients without concurrent CMV infection, one was considered to have integration and no treatment was given. The other 4 were treated resulting to death in one case (lung pathology, even though the cause of death was bacterial sepsis) and clinical improvement for the other 3 (two with encephalitis and one with marrow aplasia).

Conclusions: Low or mid levels of HHV6 may often be observed after bone marrow transplantation and they usually don't require treatment, where, high levels of HHV6 might be associated with serious clinical manifestations requiring treatment. The real incidence or the development of clinical signs might be underestimated as most of these patients receive treatment for concurrent CMV infection and potential HHV6 infection might be obscured.

P800

Voriconazole for secondary prophylaxis of invasive fungal infections in autologous stem cell transplant recipients

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Background: Recurrence of prior invasive fungal infection (relapse rate 30-50%) limits the success of stem cell transplantation. Secondary prophylaxis against invasive aspergillosis involves the administration of antifungal drugs to patiens who are undergoing a period of immunosuppression and who have a history of invasive aspergillosis.
Design and methods: Our study was conducted evaluating voriconazole (4 mg/kg/12 h intravenously or 200 mg/12 h orally) as secondary antifungal prophylaxis in autologous stem cell transplant recipients with previous proven or problable invasive fungal infection. Voriconazole was started 48 h or more after completion of condicioning chemotherapy and was planned to be continued for 40 days. Patients were followed for 12 months for incidence of proven or problable invasive fungal infections.
Results: Twenty-eight patiens were enrolled, 19 of whom had stem cell transplant for acute leukemia. Previous invasive fungal infections were proven and probable aspergillosis (n=19), proven candidiasis (n=5) and other proven or probable infections (n=2), prior infection could not be confirmed in two patiens. The median duration of voriconazole prophylaxis was 28 days.Two patiens (7%) died within 12 months of transplantation, but only one due to systemic fungal disease. Only 2 invasive fungal infections occured post-transplant: two relapses (one candidemia and combination of aspergillosis plus candidosis in 1 patient) .In one patient the prophylaxis was interrupted due to treatment related adverse events.
Conclusions: Voriconazole appear to be safe and effective for secondary prophylaxis of systemic fungal infection after high-dose chemotherapy and autologous stem cell transplantation. Treatment-related adverse events were acceptable and consistent with clinical experiences in this high- risk population. Thus, voriconazole may be a promising agent in adults undergoing SCT for a hematologic disease.

P801

Epidemiology and outcome of invasive fungal diseases in adult haematopoietic stem cell transplant recipients

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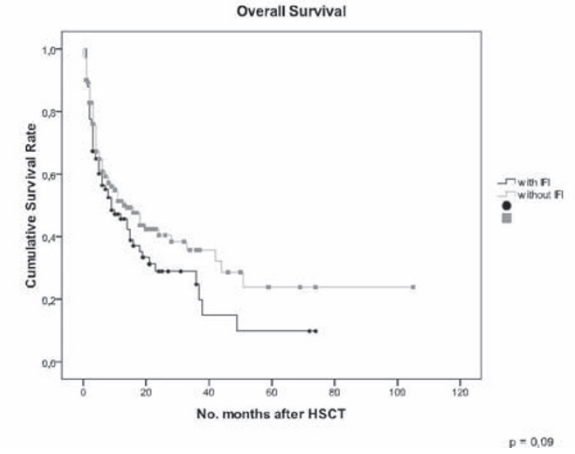
Background: Invasive fungal diseases (IFD) are lead cause of morbidity and mortality in hematopoietic stem cell transplant (HSCT) recipients. This study focuses on the risk factors, etiology and outcomes of IFD.
Methods: 356 pts after allogenic (allo) (237) and autologous (auto) HSCT (119) between 2000 through 2010 were included. The distribution of IFD and rates of overall survival (OS) at 6 weeks after diagnosis and 3 years after HSCT were studied. Baseline patient characteristics and abbreviations are outlined in table 1. International definition criteria (EORTC/MSG 2008) of IFD were used.
Results: The incidence of IFD after HSCT was 27,2% (97/356). The incidence of IFD after alloHSCT was higher 32,5% then after autoHSCT 16,8% (p<0,001). Possible IFD were detected in 30%, probable in 54% and proven in 16%, according to the criteria EORTC/MSG 2008. After autoHSCT IFD was recorded earlier then alloHSCT (p<0,001). Median date of IFD onset after autoHSCT was D+10 (6-43) vs alloHSCT–D+35 (3-610). Invasive aspergillosis (IA) 82% was the most frequent IFD, followed by invasive candidosis (IC) 12%, zygomycosis (Zygo) 4,5%, and IFD due to other molds 1,5%. The following risk factors were detected in alloHSCT recipients group: RIC, usage of fludarabin and anti-thymocyte globulin (ATG) in conditioning regimen, lymphopenia more then 3 weeks, neutropenia grade 4 more then 2 weeks, acute GVHD, previously IFI, bacterial infection, CMV infection, diagnosis AL, especially ALL, PBSC as a source of HSC (p<0,05). The risk factors in autoHSCT recipients group were: mucositis grade III-IV, lymphopenia more then 3 weeks, neutropenia grade 4 more then 2 weeks, bacterial infection (p<0,05). Unexpectedly receipt of G-CSF was associated with increased risk of IFI in both groups (p<0,001). The 3-year OS after HSCT in pts with IFD vs without IFD was 15% vs 35% (p=0,09) (figure 1). The 6-week OS was better for HSCT recipients with IA (92,5%), followed by those with IC (62,5%) and those with Zygo or IFD due to other molds (50%) (p<0,001).

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)

Figure 1. Eighty-month survival outcome after HSCT in patients with or without IFI; *p* value is calculated by log-rank test.



Conclusion: The incidence of IFD following alloHSCT was higher then after autoHSCT. We revealed difference of risk factors in allo- and autoHSCT recipients. Receipt of G-CSF was associated with increased risk of IFD in both groups. OS was decreased in pts with IFD. The 6-week survival rate was better for HSCT recipients with IA, followed by those with IC and those with Zygo or IFD due to other molds, who had the highest mortality rate.

P802

Serum GGalactomannan screening for the diagnosis of invasive pulmonary aspergillosis in children after haematopoietic stem cell transplantation and high-risk leukaemia

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Objectives: Invasive Fungal Infections (IFI) constitutes a substantial source of morbidity and mortality among immunocompromised patients. Morbidity and mortality among patients following HSCT is high. Rapid diagnosis and early treatment of IFI are crucial, yet, limited by other complications of HSCT and the need for invasive procedures mainly to identify IPA. Our study was designed to investigate the impact of serial Serum Galactomannan Assay (GMA) on the diagnosis of IPA.

Methods: Children with high risk leukemia or following HSCT were included. Serum samples for GMA (Platela, Aspergillus EIA, Biorad, France) were taken twice weekly from patients at high risk for IFI over 10 months (February-November 2010). Results over 0.5 were considered positive. Patients suspected to have IA were stratified as possible, probable and definite according to recent consensus (Clinical Infectious Diseases 2008; 46:1813–21).

Results: 27 patients were included. Median age was 11(range 1-21 years). 306 samples were processed. Of 22 HSCT children 15 were after allogeneic and 7 patients after autologous bone marrow transplantation. 5 patients had high risk leukemia. 20 patients had prolonged period of screening from 1 to 7 months. In 7 children only 1-3 samples were obtained.

GMA was negative in 14 patients; none was suspected to have IA. 13 patients had 1-4 positive GMA. Four of these 13 children (30%) had criteria of possible IPA and due to positive GMA results were upgraded to the category of probable IPA and started early antifungal treatment. However in 9/13 (70 %) of patients the test was considered false positive without any sign of IA. In our group, 6 of 13 patients (60%) showed positive Galactomannan index during high dose chemotherapy or irradiation as well as during infusion of stem cells; another 40% had positive index during Piperacillin-tazobactam treatment, engraftment period and other viral infection (EBV and HHV-6).

Conclusion: GMA may have an important role in the follow up for high risk patients and support early evaluation and treatment for IA. Negative predictive value is high among children. False positive rate is high. The cost benefit of early detection versus. over diagnosis and performing more specific tests in children should be further evaluated.

P803

Gram-negative infections and antibiotic resistance in children after stem cell transplantation: a single-centre experience

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Background: Gram-negative infections (GNI) are a major cause of morbidity and mortality after allogenic hemopoetic stem cells transplantation (alloHSCT).

Aim: Analysis of the structure, epidemiology of GNI in alloHSCT patients and evaluate antibiotic resistance of gram-negative bacteria.

Patients and methods: We performed a retrospective study between 2009 and 2010, to examine the risk factors, prophylaxis, and outcome of GNI in 120 pediatric patients at the 200th day after transplantation. Cultures were provided with BacT/Alert® and Vitek 2® technologies.

Results: The incidence of GNI was 52% of all episodes of bacterial infections (table 1), 21% developed GNI after day +60. Risk factors for GNI was disease status (relapse/>2 remission), chronic graft-versus-host disease, use of systemic steroids. All patients received as prophylaxis ciprofloxacin and 32% of isolates was resistant to this fluoroquinolone. GNI was associated with 35% dead patients. Factors associated with GNI included the presents of a copathogen and high-dose steroid use. We observed increasing rates of multiresistant strains of Klebsiella pneumoniae, Pseudomonas aeruginosa. Analysis of antibiotic resistance shows high antipseudomonal efficacy for piperacillin/tazobactam, colistin (90-100%), moderate for carbopenems (meropenem, imipenem 45-50%) and low for aminoglycosides (21%), fluoroquinolones (ciprofloxacin 15%).

Conclusions: Development of severe GNI is one of the most serious events in post transplant period. The organisms that most commonly caused GNI were K. pneumoniae, Enterobacter spp., E.coli and P. aeruginosa with increasing rates of multiresistant strains. Minimization of the use of systemic steroids may help greatly reduce the risk of the GNI post alloHSCT.

	Respiratory Tract Infections		Bloodstream Infections		Urinary tract Infections		ENT-organs Infections	
	N	%	N	%	N	%	N	%
All bacterial infections /GNI	53	46,8	80	43,75	39	92,1	25	30
Escherichia coli		5,6		3,75		25,6		12
Kl.pneumoniae		13,2		8,75		28,2		8
Enterobacter sp.		3,7		5		12,8		4
Pseudomonas sp.		11,3		12,5		17,9		6
Citrobacter sp.		5,6		1,25		-		-
Acinetobacter sp..		3,7		3,75		5,1		-
St.maltophilia		3,7		3,75		2,5		-
Other		-		5		-		-

P804

Dental status prior to stem cell transplantation: a predictor for infection - a single-centre survey

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Infections are among the most frequent and relevant complications of stem cell transplantation (SCT). Little is known about the role of the dental status for the prevalence of infections and outcome of stem cell transplantations although occult dental foci could contribute to infections in immunocompromised patients. Often screening is performed prior to SCT. Therefore, we performed a survey in all patients receiving at our center dental examinations including panoramic X-ray in preparation for SCT in the years 2003-2006, to analyze whether dental findings prior to transplantation correlate to the later occurrence of fever and infection.

163 patients, 55 female and 108 male, were analyzed with an age range from 4 to 70 years and 83 allogeneic (28 siblings and 57 unrelated donors) and 80 autologous transplants were performed. Most frequently indication for SCT was multiple myeloma (n=49), followed by AML (32), NHL (27), ALL (12), and MDS (10). Dental intervention was limited to few cases of severe dental destruction or overt dental infection. Dental findings including the presence of impacted or partially impacted wisdom teeth, teeth with root fillings as well as insufficient root fillings prior to SCT were considered as potential focus.

No dental related complications occurred during the transplantation period. Neutropenic fever was seen in 123 patients (64 allogeneic). However, we found no correlation of neutropenic fever or infections in general to the dental status prior SCT neither in the total cohort nor in the allogeneic subgroup.
In conclusion, the dental status did not predict the frequency of neutropenic fever in patients receiving stem cell transplantation. Dental surgery prior to SCT should be performed carefully and limited to those individuals with overt infection.

P805
18-Fluoro-deoxyglucose positron emission tomography as tool to evaluate and monitor disseminated fungal infections in immunocompromized patients
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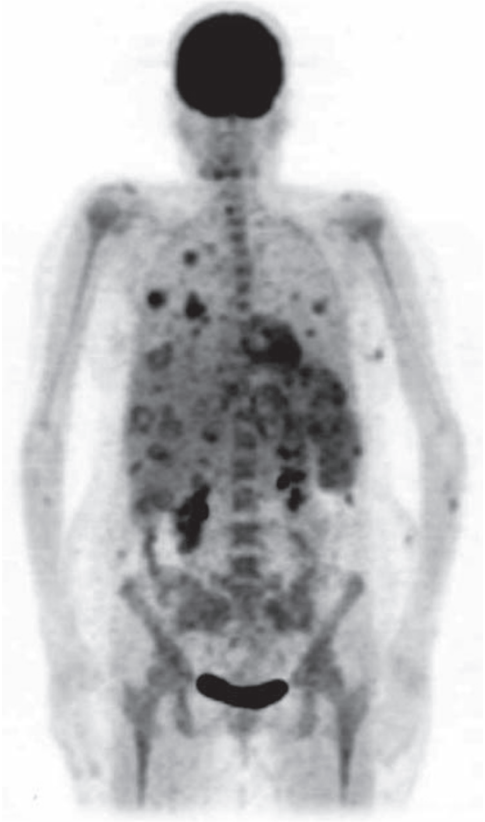
Invasive fungal infections (IFI) are a dreaded complication in the management of immunocompromised patients. A complete staging of localizations must be performed to enable an optimal surgical treatment as often as possible. It is necessary to evaluate the response treatment regularly for optimal adaptation of anti-fungal agent. Are conventional radiological methods sufficient to screen and survey disseminated fungal infections?
A 46-year-old woman received induction therapy for an acute lymphoblastic leukemia and presented a Scedosporium apiospermum disseminated infection. Computer tomography (CT) scan showed pulmonary and hepato-splenic and renal lesions. A (18)FDG-PET/CT scan confirmed the lesions and revealed a skin lesion of the left breast not detected on the CT scan (Figure1). A monthly monitoring of the treatment response by pharmacological of voriconazole and (18)FDG-PET/CT allowed the antifungal agent dosage to be adapted.



An improvement occurred on the (18)FDG-PET/CT only having reached plasma voriconazole levels above 1mg/l. A favorable outcome and complete remission have been obtain after induction treatment that was maintained nine months later with appropriate consolidation chemotherapy.
A 47-year woman with acute myeloid leukemia received induction therapy. A complete remission was obtained. The patient presented pulmonary renal thyroid and iliac lesions. Mycological examination of pelvic surgery curettage revealed a mucormycose. However an in vitro antifungal susceptibility testing could not be performed. The CT performed four months after the begining of treatment with liposomal amphotericin and posaconazole was stable but (18)FDG-PET/CT showed an aggravation. Posaconasole was stopped and caspofungin was introduced. The (18)FDG-PET/CT three months later showed an improvement with decrease of Standardized Uptake Value (SUV) but the patient dead of a relapse of the leukemia.
(18)FDG-PET/CT could also give information of aggressiveness of the infection by the description of the metabolic activity. Could (18)FDG-PET scan be an optimal technical tool to screen organ localizations and to evaluate antifungal agent response in disseminated fungal infection?

P806
Bacteraemias and invasive fungal disease infection in children with acute GvHD. A single-centre experience
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Introduction: Bacteriemias (B) and invasive fungal diseases (IFD) represent severe complications after allogeneic hematopoietic stem cell transplant (allo-HSCT). Objectives of this study are to evaluate the incidence of B and IFD in children who developed classic (C) acute GvHD vs those with the persistent/recurrent (P/R) form (according to NIH consensus), in those with refractory (R) vs not refractory (NR) aGvHD, and in pts treated with mono/polyclonal antibodies (MoAbs) vs those who received other therapies.
Patients and methods: Between January 00 and December 09, 201 children with a median age of 8,08 yrs (1°q 2,66-3°q 11,70) received 219 allo-HCST at G. Gaslini Research Institute in Genoa/Italy. The underlying diseases included acute hematological malignancies (65%), inborn errors (16%), acquired/congenital aplastic anemia (11%), hemophagocytic lymphohistiocytosis/histiocytosis (4%), solid tumors (4%). 159 HSCTs (73%) were performed using an alternative donor, and 60 (27%) a matched related donor. Conditioning regimen (CR) was myeloablative in 75% of cases, non-myeloablative/reduced intensity in 25%. Overall, aGvHD occurred in 143 HSCTs (65%), 98 with C (68%) and 45 (32%) with P/R signs.
35 pts failed 1st line treatment with methylprednisolone (MPD, 2 mg/Kg/day) R aGvHD, 14/98 pts (14%) in the C aGvHD group and 21/45 (47%) in the P/R group. 28 pts (80%) received MoAbs as 2nd line therapy (daclizumab: 9; ATG: 3; etanercept: 11; infliximab: 5). The remaining 7 pts received higher doses of MPD.
Results: Table 1 reports the incidence of B and IFD in pts with aGvHD. B were gram-positive related in 8 pts and gram-negative in 11 pts. IFD were 4 fungemia, 2 documented, 2 probable and 4 possible mycosis. Pts treated with MoAbs developed 9 B (3 after daclizumab, 6 after etanercept), and 7 IFD (6 after etanercept and 1 after infliximab). Median follow-up for C, P/R,

Table I

	Bacteriemias (n)	Invasive Fungal disease (n)	p for B	p for IFD
a-GvHD	23	12		
Classic vs persistent/recurrent	5 vs 18	3 vs 9	p<0.001 [1]	p=0.005 [2]
Refractory vs not refractory	12 vs 11	10 vs 2	p=0.04 [1]	p<0.001 [2]
Monoclonal A. vs no	9 vs 3	7 vs 3	p=0.683 [2]	p=0.421 [2]

[1] χTest chi quadro; [2] Fisher exact Test

R and NR aGvHD were 11 (6-31), 276 (122-521), 12 (7-44) and 122 (48-433) days, respectively.
Conclusions: These data show that patients with P/R aGvHD had a significant higher proportion of B and IFD than those with C aGVHD, as well as those with R vs NR aGvHD. The use of MoAbs did not significantly increased the incidence of B and IFD. The identification of groups at risk for severe infections allows us to implement the protocol of infectious surveillance.

P807

Anidulafungin primary prophylaxis in 28 high-risk patients undergoing allogeneic haematopoietic stem cell transplantation: a single-centre experience

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Background: Invasive fungal infections (IFIs) remain an important causes of morbidity and mortality for patients (pts) undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). Anidulafungin (ANI) is a new echinocandin drug with a broad spectrum of action and low toxicity profile without relevant drug interaction. According to definitions of the high risk level for IFIs in pts transplanted in our Institution, we decided to test ANI efficacy as antifungal prophylaxis in the allo-HSCT setting.
Aims: Aim of this study is to evaluate the safety and efficacy of ANI as antifungal prophylaxis in pts with high risk hematological malignancies receiving allo-HSCT.
Materials and methods: We analized 28 pts affected by high-risk hematological malignancies who underwent allo-HSCT (15 haplo, 8 MRD, 4 MUD, 1 CB) in our Institution from July 2009 to November 2010. ANI was started one day before the conditioning (200 mg die iv single dose, then 100 mg die iv) until neutrophil engraftment (PMN >0.5x10^9/l for 3 consecutive days). After engraftment, ANI was replaced with voriconazole. ANI was used like secondary prophylaxis in only one patient. Disease status at allo-HSCT was intermediate/advanced (I/A) in 18 pts (haplo: CR1=2 pts, I/A=13 pts; MRD: CR1=1 pts, upfront= 5 pts, I/A=2 pts; MUD: CR1=1 pts, upfront=1 pts, I/A= 2 pts; CB: I/A= 1 pts). 22 pts received ATG. 2 pts underwent a previous allo-HSCT. The median time from diagnosis to allo-HSCT was 552 days (92-4022).
Results: Only one allergic grade 2 skin toxicity affected 1 patient after the first drug infusion; the patient was dropped-out of the study. Median duration of ANI therapy was 24 days (9-44). Median time for neutrophil engraftment was 18 days (13-57). 13/27 pts stopped ANI at neutrophil engraftment, without signs of IFIs. 14 pts with febrile neutropenia developed IFIs (7 probable, 7 possible) and we replaced ANI with voriconazole (12 pts) or Amphotericin B Liposomal (2 pts). In the MRD setting, 6 pts stopped ANI for engraftment, 1 for possible IFI, 1 for skin toxicity. In the MUD setting, 3 pts stopped ANI for engraftment, 1 for possible IFIs. In the haplo setting, 4 pts stopped ANI for engraftment, 11 for probable/possible IFIs. Patient receiving CB stopped the drug for probable IFI.
Conclusions: ANI is a well tolerated drug. ANI prophylaxis is an effective option in the allo-HSCT setting for pts with high-risk hematological malignancies.

P808

Incidence, clinical features, and outcome of respiratory virus infections in paediatric patients after haematopoietic stem cell transplantation

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Background: Respiratory viruses (RVs) are known to be a cause of mortality after hematopoietic stem cell transplantation (HSCT), but there is paucity of study on RV infections in HSCT recipients in Korea. This retrospective study was aimed to determine the incidence, clinical features, and outcome of RV infection in pediatric HSCT patients.
Methods: Data were collected from 211 HSCT pediatric recipients who underwent transplantation during 3-year period (2007-2009) and assessed viral infection episodes by testing respiratory samples, within 28 days after HSCT. Samples were tested by culture and multiplex PCR for Influenza, Respiratory Syncytial virus (RSV), Parainfluenza (PIV), Coronavirus (CoV), Rhinovirus (RhV), Adenovirus (AdV), Metapneumovirus (MPV).
Results: Of 108 autologous and 103 allogenic HSCT recipients, 16 (13 autologous, 3 allogenic) had infection episodes caused by RSV (4), influenza (1), PIV (2), AdV (1), CoV(5), RhV (4). Among 16 patients, 1 patient who underwent autologous HSCT died from RSV pneumomia 4 days after detection of RSV. Another patient died after allogenic HSCT due to pulmonary hemorrhage derived from disseminated intravascular hemorrhage, which was probably caused by with RhV and AdV. Remaining 14 episodes were resolved spontaneously. Multiplex PCR increased the yield of positive specimens significantly compared with traditional culture methods (P<0.0001).
Conclusion: RV infections are increasingly recognized as important complications in HSCT recipients based on the recent development of rapid and highly sensitive diagnostic method such as multiplex PCR. We should raise our recognition and detect early to reduce mortality regarding the existence of RV infection in pediatric HSCT recipients.

P809

Use of micafungin in haematopoietic stem cell transplantation: a single-centre experience

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Invasive fungal infections remain one of the main causes of mortality in hematopoietic stem cell transplant (HSCT). Among these patients, micafungine has shown efficacy as antifungal prophylaxis and treatment. Micafungine is an echinocandin that inhibits the b-1,3-glucano, and thus it is active against Candida and Aspergillus. This antifungal spectrum with its safety profile makes it a good choice for hematological patients.
We assessed the efficacy and safety of micafungine in 18 patients with a medium age of 37 years (1-65) undergoing allogeneic (17; donor: 7 related, 9 unrelated and 1 cord blood) or autologous transplantation(1). Among the allogeneic transplants, conditioning regimen was myeloablative(6) and reduced intensity (11); all patients received graft versus host disease(GVHD) prophylaxis and 76% of them developed acute GVHD.
Fifteen patients received micafungine as antifungal prophylaxis. Fifty percent of them received it as initial prophylaxis in transplant neutropenia and it was maintained from day +1 to discharge. The medium days of treatment was 38 (range, 8-62). In 33%, posaconazole was switched to micafungine due to liver toxicity and in 13% amphotericin B was switched to micafungine because of a high risk of aspergillosis and contraindication for using azoles due to treatment with rapamicine. Only one of these 15 patients (receiving micafungine as antifungal prophylaxis) developed a possible invasive fungal infection (respiratory symptoms and suggestive images on CT scan) on day +37. He was treated with amphotericin B without response and passed away due to respiratory failure.
In 2 patients, micafungine was started as empirical treatment in the event of neutropenic fever not responding to broad spectrum antibiotics. Both patients completely recovered. There were no microbiological isolates.
In 1 patient, micafungine was used as targeted therapy after documenting the Candida in blood cultures. The clinical evolution of the patient was favorable and blood cultures became negative.

We did not document any adverse effects potentially related to micafungine. In patients with previous liver toxicity due to posaconazole, an improvement in liver function tests was observed.
In conclusion, from our centre experience, Micafungine is a safe and effective antifungal prophylaxis in hematological patients, and it can be an alternative for those with contraindications for using azols or who have developed adverse effects with previous prophylaxis.

P810

Cytomegalovirus infection with leukopenia especially neutropenia after allogeneic haematopoietic stem cell transplantation: the risk factor of progression from CMV infection to disease

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Introduction and objective: Cytomegalovirus (CMV) infection and disease are the major cause of morbidity and mortality in allogeneic hematopoietic stem cell transplantation (allo-HSCT). CMV infection is usually non symptomatic. If we can catch the risk of CMV disease with starting CMV pre-emptive therapy, it may contribute to effective therapy for CMV infection before the development of CMV disease. Mildly symptomatic CMV infection presents insidiously with fever, anorexia, and malaise without additional signs or symptoms. Sometimes hematologic abnormalities such as leukopenia, typically without the presence of atypical lymphocytes, and thrombocytopenia come up. The aim of this study was to investigate the risk factor of progression from CMV infection to CMV disease without response to pre-emptive therapy and the association between blood count abnormality and response to CMV infection treatment.
Method: We collected 43 patients received pre-emptive therapy with CMV reactivation detected with quantitative polymerase chain reaction (quantPCR) after allo-HSCT between 1 January 2006 and 31 August 2010.
Result: Patients with progressed to CMV disease among them are 7 (16%). Number of total episodes of CMV infection is 95. Number of episodes of CMV disease is 10 (11%). In multivariate analysis, the risk factor of progression from CMV infection to CMV disease despite the pre-emotive therapy were leukopenia especially neutropenia at the time of first detection of CMV replication (hazards ratio for leukopenia=17.84, P=0.003; hazards ratio for neutropenia=7.59, P=0.02) and the use of ATG or alemtuzumab (hazards ratio for 13.24; P=0.036). Of note, in leukopenia group, rate of failure of clearing CMV infection after one cycle of pre-emptive therapy is higher (57% vs. 10%, P<0.001). In 4 of 17 episodes of leukopenia and/or neutropenia, patients died during CMV treatment and all the rest of leucopenia and/or neutropenia were recovered average 11.2 days (range, 1-40) after first negative CMV PCR result following treatment.
Conclusion: These data show that leukopenia and/or neutropenia initially documented with CMV infection involve the virological response to pre-emptive therapy and it is the notable risk factor for progression from CMV infection to disease in addition to patient factor like poor T-cell recovery.

P811

Toxoplasmosis after allogeneic stem cell transplantation: a single-centre experience over ten years

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Toxoplasmosis is a rare and underestimated complication following allogeneic stem cell transplantation with an often fatal course. This is in part due to limited diagnostics relying mainly on imaging and molecular based techniques. In this report we present three cases of toxoplasmosis identified among 155 allograft recipients treated at Greifswald University Medical Centre. Widely disseminated toxoplasmosis was detected post mortem in two patients allografted for high-risk multiple myeloma. Clinical signs suspicious for toxoplasmosis occurred after day +32 and +75, respectively. In one case toxoplamosis was not suspected and in the other case the serology and conventional PCR revealed negative results. Both patients did not receive trimethoprim-sufmethoxazole (TMP-SMZ) but pentamidine for Pneumocystis-jirovecii-pneumonia-(PcP)-prophylaxis, The third case was a 68-year old women allografted for AML. She developed cerebral toxoplasmosis from day +395 after allogeneic SCT with typical MRT signs. Toxoplasma-DNA was amplified from 1 of 2 samples of cerebrospinal fluid (CSF). She died from progressive parasitosis despite therapy. Retrospective comparison of conventional PCR with a newly established quantitative real time PCR on clinical specimens of the three patients revealed clearly the superiority of the latter method. In conclusion, we suggest a closely rtPCR-monitoring for high-risk patients or patients with signs of infections. In general we encourage the use of TMP-SMZ instead of pentacarinate for PcP-prophylaxis, nevertheless, the efficacy of TMP-SMZ in this setting has not been proven so far.

P812

Antifungal prophylaxis with posaconazole in allogeneic stem cell transplantation

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Invasive fungal infections are an important cause of morbidity and mortality after allogeneic haemopoietic stem cells transplantation (HSCT). Posaconazole is a new generation oral azole with a wide spectrum activity and a safety profile. This retrospective study analyzes efficacy and safety of posaconazole for fungal prophylaxis in patients undergoing allogeneic HSCT in our center. We compared posaconazole prophylaxis in 23 patients (56%) undergoing allogeneic transplant since February 2008 with fluconazole/itraconazole prophylaxis in 18 (44%) allogeneic transplantated patients before February 2008.Table1 shows patients characteristics. No significant differences in clinical features was found between the two cohorts of patients. The median CD34+ cells infused was $5{,}03 \times 10^6$/Kg (range 2,01-20).The median time to platelet and neutrophil engraftment higher than 20×10^9/L and 0.5×10^9/L were 14 (range 10-33) and 12 days (range10-23), respectively. Mucosites occurred in all patients(grade III-IV in 7 patients). Thirty patients (73%) experienced a febrile neutropenia (table2). Nine episodes of fever of unknown origin were documented (30%). Two pneumonitis (7%) and nineteen bloodstream infections (63%) occurred. Three probable invasive fungal infections were reported (10%) (2 GM positive plus imaging findings and one C.Albicans sepsis). All patients received a systemic antimicrobial therapy(median day of treatment:16, range 2-42).Ten patients (33%) had a favorable response to primary antimicrobial treatment.The empiric or pre-emptive antifungal therapy was administered in 13 (43%) patients. Fewer patients (4/13) in the posaconazole group (31%) underwent the antifungal empiric or pre-emptive treatment ($p<0.05$).The median days of antifungal treatment were 10, range1-30, without statistical difference between the two groups. No difference was observed regarding the febrile neutropenia episodes, the proven fungal infections and duration of antifungal treatment between the two group of patients. TRM at +100 days was 24%, similar in the two cohorts. No adverse serious events occurred during the antifungal prophylaxis. Three patients (13%) treated with posaconazole withdrawn the drug because of gastrointestinal symptoms. In summary, prophylaxis with posaconazole is effective and safe in patients undergoing allogeneic transplant and resulted in lower rate of empiric/pre-emptive systemic antifungal treatment.

[P812]

Table 1: Patient Characteristics

Patient Characteristics	N = 43
Median age, y (range)	46 (13-69)
>55 years	12 (29%)
Primary underlying diagnosis, no. (%)	20 (46%)
Non-Hodgkin Lymphoma	5 (12%)
Hodgkin disease	3 (7%)
Multiple Myeloma	11 (26%)
Acute Leukemia	2 (5%)
Myelodysplastic disorder	2 (5%)
Other	
Median number of prior chemotherapy, (range)	2 (1-4)
>2	20 (47%)
Type of allograft donor	38 (88%)
Matched, related	3 (7%)
Mismatched, related	2 (5%)
Matched, unrelated	
Status at transplant	15 (35%)
CR	12 (28%)
PR	16 (37%)
Progression/NR	
Median time to HST, months (range)	12 (4-117)
Antimicrobial prophylaxis	28 (65%)
Chinolonic drugs	15 (35%)
Other	14 (6-32)
Median days of prophylaxis, range	
Antifungal prophylaxis	23 (56%)
Noxafil	15(35%)/3(9%)
Itraconazole/Fluconazole	21 (7-39)
Median days of antifungal prophylaxis, range	

Table 2: Results

	All patients	NOXAFIL		P
		YES (23)	NO (18)	
FEVER	30 (73%)	16 (53%)	14 (47%)	0,55
FUO	9 (30%)			
Microbiological	19 (63%)			
Pneumonitis	2 (7%)			
Median day of onset	4 (-3; +13)			
Median duration of fever	7 (1-26)			
IFI	3 (10%)	2	1	0,7
Use of systemic antifungal therapy	13 (43%)	4/13 (31%)	9/13 (69%)	0,83
Amphotericin B colloidal dispersion	5			
Liposomal amphotericin B	4			
Voriconazole	2			
Caspofungin	2			
Median days of AFT*, range	10 (1-30)	3	3	0,16
>10 days	6 (46%)			
Median time spent in Hospital, range	28 (11-59)	9	9	0,4
>28 days	18			
TRM at + 100	10 (24%)	5	5	0,69

Abbreviation AFT*: antifungal treatment

P813

Herpes simplex virus reactivation in children and adolescents with acute leukaemia after allogeneic haematopoietic stem cell transplantation: influence of conditioning regimen intensity

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Introduction: HSV is a significant infectious pathogen in allo-HSCT recipients. Organ and tissue damage, duration and intensity of immunosupression are among main triggering factors of HSV reactivation. We supposed that allo-HSCT with reduced-intensity conditioning (RIC) would decrease HSV reactivation due to reduced organ and tissue damage.
Objectives: studying incidence of HSV reactivation in allo-HSCT with myeloablative conditioning (MAC) vs RIC, and studies of correlations between HSV reactivation and incidence/severity complications in allo-HSCT.
Patient and methods. Fifty-four children and adolescents up to 21 years old with ALL and AML underwent matched related and unrelated HSCT (either bone marrow or peripheral stem cells) with MAC (n=31) and RIC (n=23) were included in the study. HSV reactivation (HSV+) was detected by means of qualitative PCR in blood, performed weekly both before and after engraftment until d+30. Toxic complications (hepatitis, neurological disorders etc) were determined according to appropriate criteria.
Results: We compared the incidence some toxic complications (mucositis, severe mucositis, hepatitis, neurological disorders), occurrence of acute graft-versus-host-disease (aGVHD), including aGVHD III-IV, intestinal aGVHD, hepatic aGVHD, incidence of infectious complications, including aspergillosis and sepsis in the following groups: “allo-HSCT HSV+” vs “allo-HSCT HSV-”, “MAC HSCT HSV+ vs MAC HSCT HSV-”, “RIC HSCT HSV+ vs RIC HSCT HSV-”, “MAC HSCT HSV+ vs RIC HSV+”, “MAC HSCT HSV- vs RIC HSCT HSV-”. Observed difference in evaluated groups haven’t any statistical significance, only a strong tend to more frequent rate of infectious complications in “allo-HSCT HSV+” group vs “allo-HSCT HSV-“ group were observed, 88% and 39% respectively (p=0,057).
Conclusion: HSV reactivation may have some relations with transplant-related morbidity, especially with other infections.

Patients characteristics

No. of patients	54
Age range (median), yrs	1-21 (12)
Number (%)of patients by parameter	
Gender	
Male	36 (67)
female	18 (33)
Underlying disease	
ALL	44 (82)
AML	10 (18)
Status of disease	
CR 1	12 (22)
CR2	22 (43)
CR3 and relapse	19 (35)
Conditioning regimen	
MAC	31
Bu 16 Cy 120 +/- ATG	23 (74)
Treo 14 Cy 120+/- ATG	8 (26)
RIC	23
Flu 180 Bu 8	7 (30)
Flu 180 Mel 160	14 (70)
aGVHD prophilaxis	
Cs MTX	32 (59)
Cs MMF	7 (13)
Tx MMF	15 (28)

P814

Impact of pre-transplant colonisation on infections profile of patients undergoing haematopoietic stem cell transplantation

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Introduction: Despite considerable progress registered in the management of hematopoietic stem cell transplantation, infection remains an important cause of morbidity and mortality.
Objectives: Therefore we aimed evaluating the frequency and type of posttransplant infections and to correlate their etiological profile with the nature of pretransplant colonization, in order to find out its predictive value for the future infectious complications.
Patients: It is a retrospective descriptive unicentric study performed on 129 consecutive auto-96 and allo–33 transplanted patients. Their pathological background consisted of

leukemia–27%, malignant lymphoma–46.5%, aplastic anemia–2.3%, solid tumors-10.85% and others–13.17%. All had an identic supportive care: primary prophilaxy with cyprofloxacine, fluconazol, TMP/SMX and acyclovir and a nursing in LAF or in HEPA filtered rooms.
Methods: A pretransplant screening for bacterial and fungal colonization and a complex bacterial, fungal and viral investigation in febrile neutropenia or symptomatic patients were undertaken.
Results and discussion: Pretransplant swabs screening revealed a colonization in 45.73% patients, 81.35% with gram positive and 18.64% with gram negative strains; fungi were isolated in 3,87% of them. Febrile neutropenia was noticed in 65.1% patients, associated with positive bacterial cultures in 47.28% patients (in blood 39.3%, urine 24.5%, catheter 16.3%, others 3.27%). Bacterial etiology was documented in 78.2%, in form of systemic (26.35%) or localized (51.93%) infection. In discordance with the colonization, the profile of agents was dominated by 65% gram negative strains in the first 5 years and 82% in the following period. Methicilin and vancomycine resistant germs were found in 42.85% of the staphylococcus infections. Fungal infections were registered in 13.17%. The registered viral infections were of exogenous (13.8%) or endogenous (86.1%) origin. The reactivation of latent infections happened in 23.25% of cases. We identified adenovirus and polyoma infections both in 1.55% of cases and reactivation of HBV infection in 3.87% patients. The mortality rate was 24.8%, infections representing 6.2%.
Conclusions: Preventive behavior, search of environmental foci of infection, awareness regarding institutional ecology and pattern of resistance, early identification of infections, a specifically tailored therapy, are only some conditions for improvement of life expectancy and increasing of cure rates.

P815
Bacterial infective complications during an early post-transplant period
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Background: infections are the most common complications of stem cells transplantation (SCT) and major cause of death together with GVHD. During an early aplastic period after SCT, bacterial infections predominate, and central venous catheter and deep neutropenia are the most important factors. Because of high rate of mortality, due to gram-negative bacteria, especially with spp. Pseudomonas, prophylaxis against this bacteria is mandatory. This strategy offer predomination of gram-positive bacteria in all sites of isolation.
Material and methods: during a period of 10 years, we have transplanted 195 patients with different hematological malignancies (AML:91 ALL:10 CML:7 CLL:1 NHL:20 HD:27 MM:34 AA:2 MP:1 CLL:1
Ewing sarcoma: 1). Allogeneic sibling transplantation: 56 Autologous SCT:139.PBSC were used as a source of stem cells in 168, bone marrow in 28 patients. Conditioning regimen consisted chemotherapy only (myeloablative in 188, RIC in 7 patients. All patients were treated in sterile rooms conditioned with HEPA filtration, low bacterial diet, and antibacterial prophylaxis with Ciprophloxacine 1000mg per day. In order to monitoring local microflora in all patients we have performed two times a week: blood cultura, urine culture, sputum, throat and nose smears and simples from central venous catheter.
Results: Gram-positive cocci were predominantly isolated bacteria from all sites. The most frequent was staphylococcus coagulasa negative, especially from CVC. Gram-positive cocci (72%), Gram-negative bacteria (18%), fungi (10%, with predominance of non-Albicans candida vs. Candida albicans 52% vs 48%). MRSA was isolated in 9,6% from all Gram-positive bacteria.Vancomicyn-resistant Enterococcus was no isolated. Distribution of infection was: bacterial pneumonia (10%), CVC associated infection: 16%, cellulitis (1%), sepsis (2%), one fatal sepsis with Stenotrophomonas maltophilia.
Conclusion: local microflora monitoring is mandatory in menagement of infections in transplanted patients and have a significant effect on the choice of empirical antibiotic prophylaxis and therapy.In our center Gram-positive bacteria clearly predominate in all isolates, but because of problems with bacterial resistance in some centers Gram-positive prophylaxis was not used in our center.

P816
Evaluation of significant clinical signs and therapy characteristics in patients with CMV infection after allogeneic stem cell transplantation
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Cytomegalovirus (CMV) reactivation is among the most important complications that determine morbidity and mortality in recipients of allogeneic hematopoietic stem cell transplants (HSCT). At the same time, due to a wide range of side effects of antiviral drugs essential is a relevant selection of appropriate therapy based on viral load and on the serological status of donor and recipient.
The aim of our research was to determine the clinically relevant parameters of CMV infection by measuring the levels and dynamics of viral load (VL) by quantitative real-time PCR for selection of optimal schemes of antiviral treatment and prevention.
Materials and methods: During the research, the analyzed samples of blood and bone marrow of 96 patients who underwent allogeneic HSCT (59 HLA-matched unrelated, 32 related and 7 from haploidentical donors). All patients transplanted from unrelated donor or haploidentical received antithymocyte globulin (ATG). The determination of VL in cerebrospinal fluid and blood plasma (with a certain number of copies of virus per 1 ml of material) as well as in bone marrow samples using commercially available kits.
Results: Reactivation of CMV infection was observed in patients in 85% of cases in the early and 15% of cases - in the late post-transplant period. Reactivation does not depend on the intensity of the conditioning regime, but was significantly higher in patients undergoing unrelated allogeneic transplantation compared with transplantation from related donors (75% and 40% respectively). Prevention of graft-versus-host with ATG was associated with an increased risk of reactivation - 70% with ATG compared to 34% without ATG. Authenticity of CMV in plasma was in direct correlation to the number of copies of viral DNA, normalized to 10^6 bone marrow cells ($p < 0,01$). Only 2 patients determined by the low titer of CMV DNA (10^3) in the bone marrow against undetected VL in plasma.
Conclusions:
1. Analysis of plasma no less informative than the study of bone marrow and shown in the monitoring of infection during the cytopenia period.
2. Late reactivation of CMV infection occurs much less frequently than earlier.

P817
Bilateral loss of vision following allogeneic haematopoietic stem cell transplantation
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Objective: We report a case of acute loss of vision six months after allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT).

Methods: A 17-year-old girl with high risk acute myeloid leukaemia, received an allo-HSCT from a HLA-compatible sibling donor. One month after allo-HCST the patient developed acute Graft versus Host Disease grade III (aGvHD) of skin and gut, responsive to steroids, tacrolimus, mycophenolate mofetil (MMF) and extracorporeal photoapheresis. Immunosuppression was continued with tacrolimus. Three months after allo-HSCT she presented rhinorrea: X-ray SENI was negative for sinusitis. Three months later, the patient presented with sudden onset bilateral loss of vision, which partially improved with topical steroids. Two months later the vision worsened and mild sporadic headache appeared. Ophtalmoscopic evaluation, Flash Visual Evoked Potential (FVEP) analysis were performed in addition to cerebral and maxillo-facial Magnetic Resonance Imaging (MRI) studies and Computerized Tomography scannings (CT).
Results: Ophtalmoscopic evaluation showed a picture of bilateral optic neuritis, confirmed by maxillo-facial MRI. FVEP demonstrated ab-extrinseco compression of optic nerve, and cerebral CT scan and MRI showed pansinusitis. Diagnosis of Orbital Apex Syndrome (OAS) secondary to pansinusitis was done. Intravenous large spectrum antibiotics and antifungal therapy were started, without any visual recovery. Surgical drainage with orbital decompression were performed, resulting in dramatic clinical improvement. Bacterial and fungal cultures were negative. Eight months after OAS' diagnosis the patient doesn't show visual impairment, with complete resolution.
Conclusion: Acute inflammatory optic neuritis maybe due to GvHD following allo-HSCT. However a number of other conditions maybe associated with optic neuritis, such as OAS due to infections. The prompt diagnosis and early treatment, especially surgical drainage, may prevent irreversible visual loss in the affected eye.

P818

Invasive fungal infections during a construction period

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Fungal infection with an incidence about 15%; remains a major cause of infectious death in the allogeneic stem cell transplantation. The risk has considered to be lower in the autologus transplantation. We aimed to find the fungal infection rates during the construction of another building near a transplantation unit and asess the outcomes of antifungal prophylaxsis.
Sixty four autologus and thirty four allogeneic transplantation patients were evaluated respectively. In the autologus group, Female/Male: 23/41, median age was 51 years (36-70). Diagnosis of patients were Non Hodgkin lymphoma/Hodgkin lymphoma/multil myeloma; 11/6/47 respectively. Patients were given amphotericin deoxycholate (0.3 mg/kg) for five months after the construction and fluconazole (200 mg) in the rest 3 months.
In the allogeneic transplantation group Female /Male:15/19 Median age was 30 (19-58)years. Diagnosis of patients were Acute myeloblastic leukemia/acute lymphoblastic leukemia/ Myelofibrosis/myelodisplastic syndrome/Hodgkin/Aplastic anemia/Paroksismal nocturnal hemoglobinuria: 18/7/2/1/1/2/2/1. Five patients had unrelated HLA matched peripheral stem cell transplantation and three patients had unrelated cord blood transplantation. Six patients were given secondary prophilaxysis with voriconazole. The rest of were given amphotericin deoxycholate(0.3 mg/kg) as primary propfilaxysis for 8 months after the construction.
In the autologus patients group only two (3%) patients were diagnosed as invasive pulmonary aspergillus infection by computerized tomography; one in the amphotericin group and the other in the fluconazole.
In the allogeneic secondary prophilaxysis group, one patient had progressive fungal infection and died because of bacterial sepsis, respiratory failure. In the primary prophylaxsis group five patients (17%) diagnosed as invasive fungal infection during the follow up. Median time from hospitalization to diagnosis were 30 days and median time from transplantation to diagnosis were 16 days.
We showed that during the construction period amphotericin prophylaxsis must be given to allogeneic transplantation patients but it has no superiority for autologus patients. It has to be decided by the physician according to patient characteristics.

P819

Pulmonary mucormycosis

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Introduction: Mucormycosis, also known as zygomycosis, is a rare opportunistic fungal infection that mostly affect immunocompromised patients. main clinical presentation is pulmonary infection that is often indistinguishable from pulmonary Aspergillosis but doesn't respond to tretment with voriconazole.
Case report: The authors report the case of a 12 years old boy who underwent bone marrow transplantation for aplastic anemia, that since 6th day post transplantation showed an x-ray opacity in the upper lobe of the left lung. Despite broad spectrum antibiotics combined with an antifungal agent (liposomal amphotericin B) there wasn't improvement. Chest computed tomography showed a cavitated nodule contacting the left apicl pleural suggesting fungal infection, probably aspergillosis. Voriconazole was started but the radiological image persisted and a month later was performed a left upper lobectomy. Histopathological examination of surgical specimen revealed a mucormycosis. After surgery this boy was treated with liposomal amphotericin B replaced 6 weeks later by oral posaconazol,with good clinical and radiological response.
Discussion: This case illustrates the dificulties of differential diagnosis between pulmonary aspergillosis and mucormycosis. The first choice antifungal therapy is amphotericin B. Posaconazole has been used in some cases that didn't respond to amphotericin B. In most patients surgical debridement of necrotic tissues that not allow antifungal agent penetration is essential.
In patients receiving immunossupressor agents secondary prophylaxis shoul be discussed.

P820

Rhinocerebral mucormycosis in a transplanted patient with relapsed acute lymphoblastic leukaemia: a case report

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Background: Mucormycosis is a rare and serious opportunistic infection caused by fungi of the order Mucorales, predominantly seen in immunocompromised patients. Ketoacidosis, neutropenia, steroid therapy and hematological malignancies are the main factors making one susceptible to this infection. In majority of cases clinical presentation is rhinocerebral mucormycosis and, if not diagnosed promptly and treated adequately, outcome can be fatal. We present a patient with this rare fungal infection, previously treated with allogeneic hematopoietic stem cell transplantations.
Case report: A 37 year old woman was admitted in our center because of 4th relapse of her acute lymphoblastic leukemia (ALL) (Table 1). For this relapse she received combination chemotherapy and anti-infective prophylaxis (Table 2). Ten days following chemotherapy she became febrile. Microbiological cultures, cytological smears and Galactomannan tests were done repeatedly, but did not reveal the cause of infection. She remained febrile despite several lines of empirical antimicrobial therapy. CT-scan of lungs showed a nodal leason in the lower right lobe suggesting possible aspergillosis, so voriconazole

[P820]

Table 1. Patient's medical history

2006		2007	2008	2009		2010
August	*August – December*	*January*	*January*	*September*	*October – November*	*End of January*
Dx: ALL -FAB : L2 -WHO: B-precursor	Induction: **Hyper-CVAD** (4 cycles)	**allo-HSCT (1)** MCR (Bu-Cy)	**allo-HSCT (2)** RIC (Flu/Mel/ATG)	**allo-HSCT (3)** RIC (Flu/Mel/Campath®)	**"Pulse" steroid therapy**	**4th relaps of ALL** -Extramedullar (without PB, BM or CSF involment)
				Probable IPA (treated with voriconazole)		
		Arterial hypertension (well controlled with antihypertensive therapy) →				
						Diabetes mellitus, steroid-induced (treated with insulin) →

ALL – acute lymphoblastic leukemia; allo-HSCT – allogeneic hematopoietic stem cell transplantation; MCR - myeloablative conditioning regimen; RIC – reduced intensity conditioning; IPA – invasive pulmonary aspergillosis; PB – peripheral blood; BM – bone marrow; CSF – cerebrospinal fluid

Table 2. Course of treatment

DRUG	DOSE	DAYS OF APPLICATION
		1 · 2-3 · 4-5 · 10 · 11 · 20 · 24 · 28 · 30 · 37 · 78
clofarabine	20 mg/m²	
idarubicin	6 mg/m²	
methylprednisolone	1 mg/kg	
ciprofloxacin	500 mg bid	
acyclovir	400 mg q8h	
posaconazole	200 mg q8h	
co-trimoxazole	960 mg x2 weekly	
piperacillin/tazobactam	4.5 g q8h	
imipenem/cilastatin	1 g q8h	
cefepime	2 g q8h	
vankomycin	1 g q8h	
voriconazole	(1st day: 6) 4 mg/kg	
amphotericin B	"step-up" dosing regimen*	

*1st day: 60 mg | 2nd day: 100 mg | 3rd day: 150 mg | 4th day: 200 mg | 5th day untill end of treatment: 250 mg

was added, but without clinical improvement. After patient started to complain of nasal congestion, left upper jaw teeth-pain and left facial paresthesia, with left facial edema, nasopharinx swab was done and Rhizopus oryzae was isolated. CT-scan of paranasal sinuses showed mucosal infiltration in left paranasal sinuses without signs of intracranial progression. Due to low platelets, surgical treatment of this invasive fungal infection was contraindicated and treatment with amphotericin B (amB) colloidal dispersion was initiated. On this therapy patient started to improve clinically and soon thereafter became afebrile. After 6 weeks on amB, she was remarkably better, with marked radiological regression of rhinocerebral mucormycosis.
Conclusion: In spite a number of adverse factors such as ALL, neutropenia, diabetes mellitus, steroid therapy, the outcome of treatment in this patient was favorable. We conclude that close clinical follow-up, rapid and targeted diagnostic procedures followed by appropriate antifungal therapy can lead to good clinical responses, even in transplanted patients with poor prognosis.

P821
CMV encephalitis as late fatal event in allogeneic haemopoietic stem cell transplantatation after alemtuzumab and rituximab treatment
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We report on a case of fatal CMV encephalitis in a high-risk patient, serologically positive for citomegalovirus (CMV), who underwent hemopoietic stem cell (HSC) transplantation from a CMV-negative, unrelated donor. The patient received conditioning with alemtuzumab and post-transplant treatment with rituximab for immune thrombocytopenic purpura (IPT). On the onset of encephalitis, severe T and B immunodeficiency was documented. High values of CMV copies in the cerebral spinal fluid (CSF) and magnetic resonance (MR) brain imaging confirmed a diagnosis of citomegalovirus.
Neurological symptoms and radiological images had progressively worsened throughout the antiviral treatment which was comprised of ganciclovir as first-line and foscavir associated to CMV-immunoglobilins as second-line therapy. The antiviral therapy only controlled CMV viremia without any significant change in CMV copies into the CSF.
In the high-risk patient, a reconstitution of CMV immunity using adoptively transferred CMV-specific T cells, or a new strategy for CMV surveillance and therapy should be considered.

P822
A case of high-dose oseltamivir treatment in a patient with severe graft-versus-host disease who infected influenza A (H1N1)
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Influenza A (H1N1) infection has been noted to be common in the young and high-risk groups for influenza infection, including transplant candidates and recipients. However, optimal dosage and duration of oseltamivir for severely immunocompromised patients has not been defined. We report a case of a patient infected with influenza A (H1N1), who suffered from

skin and lung graft versus host disease after he had received allogenic hematopoietic stem cell transplantation from matched sibling donor because of relapsed neuroblastoma. During immunosuppressant therapy, he was diagnosed with influenza A (H1N1) infection by RT-PCR (real time polymerase chain reaction). He recovered after oseltamivir treatment with dosage of 90mg twice a day which was two times of the standard dose per oral until influenza A (H1N1) RT-PCR has proved negative.

Regulatory issues

P823

Association of health care expenditure with results of alloHSCT in Europe

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In a recently published study we demonstrated that results of alloHSCT in Europe are associated with the socio-economic status (SES) of a country (Giebel et al., Blood 2010). We used the human development index (HDI) as a surrogate marker. In the current analysis we looked for more detailed aspect of the SES, i.e. health care expenditure (HCE), including the contribution of public and private funds. Transplantations from HLA matched sibling or unrelated donors for patients with AML in CR1 were used as a probe. AlloHSCTs using cord blood, T-cell depletion or reduced intensity conditioning were excluded.
The total of 1849 alloHSCT procedures (sibling, n=1218; URD, n=631) performed between 2004-2008 in 21 European countries were analyzed. Median recipient age was 42 years (18-71 years). Countries were divided by quintiles and compared according to increasing values of the current HCE per inhabitant, public HCE, private HCE, and HCE as % of gross domestic product (GDP).
With the median follow-up of 24 months, the 2-year probabilities of LFS for groups of countries with increasing current HCE per capita were 62%, 58%, 63%, 59%, and 70%, respectively. LFS for countries with the highest current HCE (5th quintile) was significantly higher compared to the remaining ones (70% vs. 60%, p=0.009). The impact on LFS remained independent (HR=1.56, p=0.004) after adjustment to other prognostic factors, including HDI, and was particularly pronounced in a subgroup of siblingHSCT (HR=1.75, p=0.004). The incidence of relapse was reduced in countries with the highest current HCE (16% vs. 22%, HR=0.58, p=0.01), while no significant difference was found with respect to non-relapse mortality (14% vs. 17%, p=0.29). Both public and private HCE contributed to the global effect on outcome, however, when analyzed separately their impact did not reach statistical significance. As well, there was no significant association of HCE as % of GDP with results of alloHSCT. The comparison of patient and transplant characteristics in countries with the highest HCE and the remaining ones revealed no significant differences except for more frequent use of peripheral blood in the upper HCE group, which, however, was found an adverse prognostic factor in the Cox model.
We conclude that health care expenditure is strongly associated with the outcome of alloHSCT in European countries. This fact should be considered for the interpretation of future analyses.

P824

Rome Transplant Network, a metropolitan haematopoietic stem cell transplant programme

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Rome Transplant Network (CIC 756)is a Metropolitan Transplant Program established as a cooperative network among 7 Institutions. The RTN structures are represented by Units of Hematology, Cell Collection and Cell Manipulation. RTN Objectives: (1) to standardize transplant procedures; (2) to improve quality of transplant care; (3) to extend the potential of transplant activity over the metropolitan aerea; (4) to share expertise and professional education among healthcare providers; (5) to promote excellence of single transplant Centers; (6) to rationalize cost-management of public health. RTN is an innovative entity, which follows rules and standards established by JACIE accreditation program. RTN is structured on 3 levels: (1) the Director responsible for the whole Transplant Program and the headed staff represented by Quality Management and Data Management Offices. (2) the Boards including Directors of each clinical, cell collection,cell manipulation Units and nursing staff. The Boards is in charge of approving documents and quality assurance related to each facility, of sharing and developing clinical protocols and of evaluating personnel and its continuous training. (3) medical,biological and nursing individuals involved in each Unit. Documental System. The RTN documental system reflects the complexity of the organization. There are two categories of documents: a) newly developed documents edited by the Board and b) already existing documents within the individual Centers to be validated by the Board. A Quality Plan for every Board and a Quality Plan for the whole transplant program have been produced. Computer Platforms play an extremely important role for either the rationalization of activities or the management of information. Two computer programs have been implemented: one for the management of cell products in all stages of the process: collection, manipulation, storage and infusion and the other one allows the sharing and spreading of documentation among all the RTN Units, the management of a common database of transplant patients and the monitoring of clinical studies. RTN is a stepwise process: 4 Clinical Units, 2 Cell Collection Units and 1 Cell Manipulation Unit have firstly applied for JACIE and National Authority accreditation. In 2010, RTN registered >200 transplanted patients. Conclusions. RTN represents a major innovation in the organization of transplant activity aimed at improving the single center excellence and the quality level of public health.

P825

Economic analysis of haematopoietic stem cell transplantation in Taiwan: application of National Health Insurance Research Database

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Objective: In Taiwan, the National Health Insurance (NHI) enrolled 99% of the 23 million population, and reimbursed the medical costs of hematopoietic stem cell transplantation (HSCT). This retrospective analysis is to understand the medical utilization of HSCT in Taiwan.

Methods: All patients whose medical records fulfilled the selection criteria of HSCT from the NHI database between 2003 and 2006 were enrolled. The demographics, medical costs during the first one year after HSCT were collected and analyzed. The survival status and causes of death were cross-checked from the database of death statistics of Department of Health of Taiwan through Dec. 31, 2006.
Results: A total of 790 HSCT, 572 (72%) allo-HSCT and 218 (28%) auto-HSCT, were performed in 14 medical centers and 2 regional hospitals in Taiwan between 2003-2006. The estimated annual number of HSCT was 87 per 10 million residents, significantly lower than neighboring Asian countries with similar socioeconomic status and incidence of hematological malignancies. Acute myelogenous leukemia (25%) was the major indication of transplantation, followed by lymphoma (20%) and acute lymphoblastic leukemia (13%). Table 1 shows the characteristics of patient and costs of HSCT. Pharmaceutical costs was the major spending both within 100 days and within 1 year of transplantation. The medical costs of HSCT were significantly lower (35-36%) while the hospitalization days much longer when compared with USA (Table 1). The estimated overall survival at 100 days and 1 year after auto-HSCT was 91% and 76% respectively, and 80% and 57% after allo-HSCT.
Conclusions: The resuslts shows that the National Health Insurance's low subvention may hinder the utilization of HSCT. Nevertheless, because of this unique insurance system, the general costs of HSCT was much lower than the Western countries without sacrificing the transplant outcome. Future incorporation with national HSCT registry is warranted for detailed analysis.

Table 1. Patient characteristics and costs of transplantation

Variables	Autologous, N=218	Allogeneic, N=572	Dana-Farber[1], N=315
Gender (M/F)	113/105	321/251	184/131
Age at HSCT, median (range)	42 (2.2 – 72)	28 (0.4 – 64)	42 (33-48)
Total costs 0-100 days*	21,095	36,988	102,574
Inpatient costs*	19,794	35,694	96,264
Outpatient costs*	705	971	4,589
Hospitalization days	38	57	36
Total costs first year*	23,909	45,469	128,800
Inpatient costs*	21,277	41,967	110,082
Outpatient costs*	1,972	2,692	10,493
Hospitalization days	42	72	39

[1]Saito et al. *BBMT* 2008;14:197-207.
*The median costs were expressed with $USD

P826
Variations in the efficiency of donor searches between international unrelated blood and marrow donor registries
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WMDA and BMDW list almost 100 registries providing volunteer unrelated donors (VUD). As a user of these registry services we are interested in variations in the efficiency of services in order to optimise our search process and maximise our ability to transplant patients in a timely manner. Since 2004 we have followed the progress of VUD searches for 193 patients. After high resolution typing of the patient, our initial request goes to our national hub to provide a list of possible donors by degree of match and registry. Time to donor identification is heavily influenced by the transplant centre's own definition of 'urgency' so we studied less subjective measures such as the availability of, time to obtain, and value of (as defined by degree of matching), confirmatory samples in addition to normal measures of the quality of the received product. We requested samples from 1226 donors with a, median of 7 requests per patient (1-20). We utilised 30 registries although 50% of the requests went to only 5 registries with a median of 220 R/R, range 43-387. 806 confirmatory samples were received from 1226 requests (66%). Donor availability and the time to sample arrival were compared in the 5 most frequently utilised registries, A-E. The percentages of the requests resulting in samples and of samples found to be HLA-matched ranged from 31-76% and 28-53%. The chance of obtaining a suitable HLA-matched donor was highest from registry D at 31% and lowest from registry E at 15%. Failure to supply confirmatory samples due to inability to contact/unavailability of the donor and was unacceptably high in registry E at 47%. The median times from request to sample arrival ranged from 15-22 days. 61 patients have been transplanted and no differences were found between registries in; hours to infusion, viability at infusion, volume, total nucleated or CD34+ cell numbers, or time to engraftment. A wider evaluation of the products obtained from the 4 most frequently utilised registries did show significant differences in the numbers of CD34+ cells collected. The costs of obtaining samples varies from approximately 500-2000 Euro. Cost is not recouped in the UK if the transplant is not performed. Following the audit we have refined our search process using the initial search as a surrogate for the ease of finding a donor subsequently. Registries also have a responsibility to improve their service and minimise costs by focussing on donor retention and high resolution typing.

P827
Clinical grade isolation of regulatory T-cells from G-CSF mobilized PBSC improves with initial depletion of monocytes
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We have recently demonstrated that G-CSF mobilized peripheral blood stem cells (PBSC) CD4+CD25+FoxP3+ cells (Tregs) prevent anti-CD34+ hematopoietic stem cells Tcell alloreactivity in-vitro and co-transplantation of CD34+ cells and Tregs doesn't affect human stem cell engraftment in NOD/SCID mice. Since only a small number of Tregs can be isolated from normal peripheral blood we examined whether PBSC can be a useful source of Tregs for future clinical trials. Five leukapheresis products from healthy donors who received rh G-CSF at 10 ug/kg daily for 5 days were processed using the CliniMACS instrument (Miltenyi Biotec, Auburn, CA). We have initially isolated CD34+ cells. To isolate Tregs a 2-step procedure was initially utilized. A cocktail of clinical grade CD14, CD8 and CD19 reagents was mixed with the CD34- cells and depletion of monocytes, cytotoxic Tcells and Bcells was obtained by using the Depletion 2.1 program. The CD4+ cells were then enriched in Tregs by positive selection of CD25+ cells using a clinical grade CD25 reagent. Because PBSC contain large amount of myeloid cells, and particularly monocytes, this clinical scale 2-step strategy was compared with a 3-step method that included an initial negative selection of CD14+ monocytes, followed by negative selection of CD8+ and CD19+ cells and a positive selection of CD25 cells. Prior to isolation, the average proportion of CD4+CD25+ cells in PBSC was 0.77±0.26% in 5 separate PBSC products. After the 2-step process the proportion of CD4+CD25+ cells was 35±33% (n=3) vs 72±1% after the 3-step process. Therefore, utilizing the 3-step approach a better yield of Tregs was observed (10 vs 60%). Intracellular expression of FoxP3 was on average 74% in CD4+CD25+ cells obtained with a 3-step process. Contamination of different cell subsets in the final products enriched in Tregs was largely superior following the 2-step as compared to 3-step isolation method. Contaminating monocytes, CD8 and CD19 cells were, on average, 43 vs 5.7%, 12 vs 1.7% and 0.9 vs 0.3%, respectively. In the two procedures using a 3-step approach the final absolute number of Tregs isolated from products containing on average 30 x 10^9 mononuclear cells, was 95 and 93 x 10^6, respectively. These findings obtained using clinically available reagents and device, suggest that depletion of monocytes may improve the purity of Treg cell population isolated from PBSC. PBSC may represent a valuable source of Tregs for future clinical trials.

P828

Non-compliant activities' report: a cultural revolution in the work process

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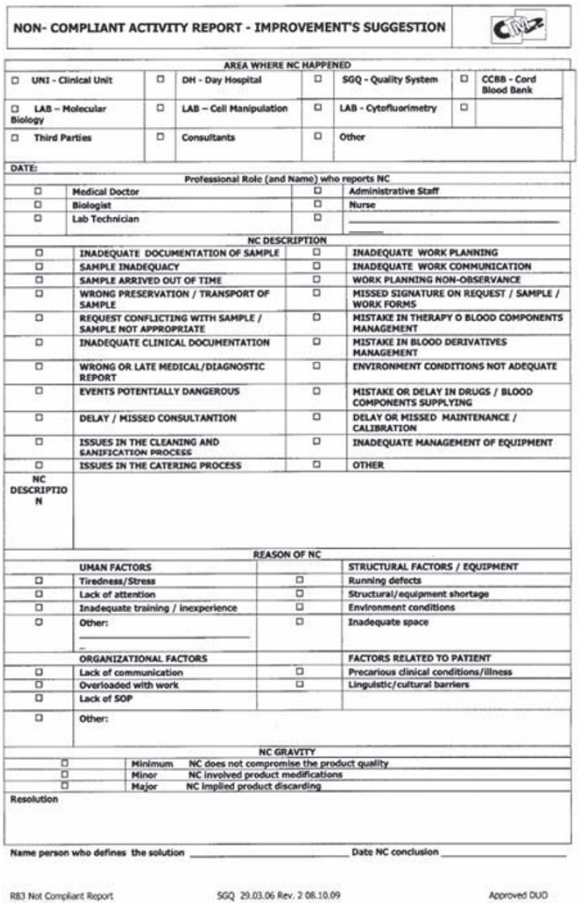
The Regional Bone Marrow Transplant and Cell Therapy Centre "Alberto Neri" (CTMO) began implementing the Quality Management System (QMS) in 2006. In September 2007 it obtained ISO certification and in 2009 applied for JACIE accreditation. From the beginning, QMS Team asked CTMO staff to report all the critical and non-compliant situations. For this purpose, we created a registration form called "Non-compliant activity/ Improvement's suggestion" (NC). The implementation of the NC forms was, first of all, a cultural revolution in the work environment. Staff's acceptance was major barrier as there was no precedent for staff taking the responsibility to report errors. The NC form is filled out in all CTMO areas (clinical unit, collection and processing facility, cord blood bank), by medical doctors, nurses, biologists, lab technicians and administrative staff. The NC form includes all work aspects: clinical, organizational and structural and it is used for internal issues and with third parties (other Hospital Departments, suppliers, etc.). From 2006 to 2010, n. 272 NC have been collected. Only in 2010 (data updated to November), n. 95: of them, 34% happened in the clinical unit and 27% are towards third parties. Medical Doctors and Nurses are the professional categories that most frequently report NC. Regarding the NC's area, 34% are related to "communication and work planning" while only 3% are "events potentially dangerous" related to dysfunction of chemotherapy kit. Doctors address NC to third parties (47%), compared to nurses (80%) and lab staff (54%) who address NC to their internal organization. From an internal survey carried out in 2009, 33% of the CTMO staff think that NC reporting is useful to clarify critical situations and 36% think that it is useful to resolve them; 37% of nurses, the people closest to patients and often the most critical, agree with this answer. The most difficult aspect is to address NC within the Hospital organization. Thanks to this tool, in the past 5 years we started to interface with the Hospital management and our NC have been used as an opportunity to identify and deal with issues common to other departments. In analyzing the NC reported so far and the response to them by the CTMO staff, we concluded that NC reporting was not easy at the beginning because it aims to identify errors and we must be critical of ourselves, managers and the organization, however it remains a helpful tool to collaborate on solutions.



NON- COMPLIANT ACTIVITY REPORT - IMPROVEMENT'S SUGGESTION

AREA WHERE NC HAPPENED

☐	UNI - Clinical Unit	☐	DH - Day Hospital	☐	SGQ - Quality System	☐	CCBB - Cord Blood Bank
☐	LAB – Molecular Biology	☐	LAB – Cell Manipulation	☐	LAB - Cytofluorimetry	☐	
☐	Third Parties	☐	Consultants	☐	Other		

DATE:

Professional Role (and Name) who reports NC

☐	Medical Doctor	☐	Administrative Staff
☐	Biologist	☐	Nurse
☐	Lab Technician	☐	_____

NC DESCRIPTION

☐	INADEQUATE DOCUMENTATION OF SAMPLE	☐	INADEQUATE WORK PLANNING
☐	SAMPLE INADEQUACY	☐	INADEQUATE WORK COMMUNICATION
☐	SAMPLE ARRIVED OUT OF TIME	☐	WORK PLANNING NON-OBSERVANCE
☐	WRONG PRESERVATION / TRANSPORT OF SAMPLE	☐	MISSED SIGNATURE ON REQUEST / SAMPLE / WORK FORMS
☐	REQUEST CONFLICTING WITH SAMPLE / SAMPLE NOT APPROPRIATE	☐	MISTAKE IN THERAPY O BLOOD COMPONENTS MANAGEMENT
☐	INADEQUATE CLINICAL DOCUMENTATION	☐	MISTAKE IN BLOOD DERIVATIVES MANAGEMENT
☐	WRONG OR LATE MEDICAL/DIAGNOSTIC REPORT	☐	ENVIRONMENT CONDITIONS NOT ADEQUATE
☐	EVENTS POTENTIALLY DANGEROUS	☐	MISTAKE OR DELAY IN DRUGS / BLOOD COMPONENTS SUPPLYING
☐	DELAY / MISSED CONSULTANTION	☐	DELAY OR MISSED MAINTENANCE / CALIBRATION
☐	ISSUES IN THE CLEANING AND SANIFICATION PROCESS	☐	INADEQUATE MANAGEMENT OF EQUIPMENT
☐	ISSUES IN THE CATERING PROCESS	☐	OTHER

NC DESCRIPTION

REASON OF NC

	UMAN FACTORS		STRUCTURAL FACTORS / EQUIPMENT
☐	Tiredness/Stress	☐	Running defects
☐	Lack of attention	☐	Structural/equipment shortage
☐	Inadequate training / inexperience	☐	Environment conditions
☐	Other: _____	☐	Inadequate space
	ORGANIZATIONAL FACTORS		**FACTORS RELATED TO PATIENT**
☐	Lack of communication	☐	Precarious clinical conditions/illness
☐	Overloaded with work	☐	Lingualstic/cultural barriers
☐	Lack of SOP		
☐	Other:		

NC GRAVITY

☐	Minimum	NC does not compromise the product quality
☐	Minor	NC involved product modifications
☐	Major	NC implied product discarding

Resolution

Name person who defines the solution _____ Date NC conclusion _____

R83 Not Compliant Report SGQ 29.03.06 Rev. 2 08.10.09 Approved DUO

P829

Evaluation of the implementation of JACIE standards by the personnel involved in a haematopoietic stem cell transplantation unit

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Background and objectives: The implementation and maintenance of a quality program in medical and laboratory practice in hematopoietic stem cell transplantation (HSCT) requires the participation of all staff members involved. This study analyzes the overall assessment of quality management program (QMP) by the professionals involved in a clinical HSCT program.

Material and methods: Between April and May 2010 a 21-question anonymous questionnaire was distributed to all staff members (medical and non-medical) involved in the clinical HSCT program. The questionnaire had 18 items involving aspects of the QMP (scored from 0 to 3 points). In addition 3 questions exploring the degree of knowledge about JACIE accreditation system were formulated (date of first audit, version of JACIE standards currently used and date of re-accreditation of the HSCT unit).

Results: Sixty-one of 84 (73%) professionals completed the survey. Seventeen (28%) were attending physicians (MD), 27 (44%) nursing graduates (NG) and 17 (28%) were support services staff (SS). The median age was 36 years [range, 21-60], 10 males and 51 females.

Ten MD (59%), 7 NG (26%) and 4 SS (15%) have reported and/or created 1 to 10 quality reports and 6 MD (35%), 8 NG (30%) and 4 SS (15%) have participated in the preparation of documents of QMP. Questionnaire results are presented in Table 1. In addition, a better knowledge by NG was found about the date of the first audit, the version of JACIE standards currently used or the date of the re-accreditation of the HSCT unit.

Conclusions: All professionals involved in the HSCT considered quality very important in their daily task and their appreciation

TABLE 1.

	Attending physicians	Nurse graduates	Supportive staff
Importance of quality management in daily activity	2,65	2,85	2,71
Importance of the quality management	2,53	2,85	2,71
QMP impact on the global clinical activity	2,35	2,30	2,24
QMP impact on patient care	2,59	2,37	2,35
Information received about JACIE	2,25	2,37	2,65
Training received after the implementation of the QMP	2,31	2,37	2,41
Improvement in the communication between professionals	2,24	2,26	2,19
Improvement in the knowledge and training in transplant	2,29	2,11	2,38
Improvement in training in quality	2,06	2,19	2,25
Detection of areas for improvement	2,35	2,27	2,44
Error detection (E) / deviations (D)	2,41	2,26	2,44
Prevention of E / D	2,24	2,22	2,50
Improvement in documentation and records	2,47	2,33	2,25
Improvement in establishment of circuits	2,41	2,33	2,31

of JACIE QMP was positive. The weakest point for MD was the need of more training in quality management, while for NG and SS was the need of specific training in HSCT. This questionnaire has identified the main improvement requirements by staff members in a clinical HSCT program.
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P830

The effect of JACIE standards implementation on stem cell storage policy

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Autologous peripheral blood stem cell transplant (aPBSCT) implies the infusion of previously collected and cryopreserved stem cells (CSC). This approach requires the storage of stem cell until transplant procedure but, sometimes, CSC are not infused: in case of death of patient before transplant, in case of patients lost to follow up, in case of no further clinical need (change of indication), in case of collection exceeding requested cell dose. The former case usually entails a CSC elimination. The other cases constitute a problem for medical and legal reasons because prior to CSC elimination, it is essential to document that the conditions for disposal have been met and are not in contradiction with consent forms signed at the time of collection. We report results of uncontrolled CSC storage policy from 1990 to 2010, after 2 years of JACIE standards implementation at our Center. During the last 20 years 1274 patients affected by haematological malignancies or solid tumors collected 3254 CSC bags; 1761 CSC bags were thawed and infused for aPBSCT; 275 CSC bags were discarded after patient death. Therefore there are 1215 CSC bags deriving from patients alive or lost to follow up; only few patients treated in the early '90 are really lost to follow up while more than 70% are alive without further indication to aPBSCT. More than 50% of the CSC bags have been stored for more than 10 years. During the years, the rate of infused bags increased: 45% in 1996-1998 and 65% in 2005-2007. This result, obviously, implied a decrease in the number of CSC bags stored and not infused per year: 46% in 1996-1998 and 27% in 2005-2007. Despite this trend our data clearly show the urgent need for strict criteria for cell dose collection and a clear policy describing conditions for maximum product storage time and/or disposal. In order to meet JACIE standards, we defined in 2008 cell dose request and adopted a new discarding policy fixing strict chronological criteria to discard unused CSC. The introduction of JACIE standardization produced a sensitive reduction in number of bags collected per patient from 1.99 in 2005-2007 to 1.69 in 2008-2010. After infusion of CSC collected in 2010 a further decrease is expected in the rate of the bags not infused also in 2008-2010. Our data show the benefit of standards implementation in the management of collection, infusion and discarding CSC bags underlying the need of shared criteria in order to ameliorate resources utilization.

P831

The central role regarding HPC transport of the unique processing facility in a multi-centre programme following JACIE standards

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Introduction: In Rome three major hospitals are part of a multi-centre Hematopoietic Progenitor Cells (HPC) transplant program (TP), called "Rome Transplant Network" (RTN). The hospitals involved are Paediatric Hospital "Bambino Gesù", University Hospital "Policlinico Tor Vergata" and University Hospital "Campus Biomedico". Other Hospitals will join the program in future. RTN is following Joint Accreditation Committee ISCT-EBMT (JACIE) standards. In the actual scenario there is one Processing Facility (PF) that performs activities for RTN and in service, for other programs located in Rome. The amount of HPC products processed yearly is about 400, and will grow in future. Non contiguous TP Facilities lie in a 22 Km wide area and the shipping between them is carried out by a courier. Within such distances in a traffic congestioned city, efficiency of shipping time makes the difference in the TP outcome.
Objective: Describe organizational changes in a multi-centre program by adopting JACIE standard.
Methods: Decision making in defining how to achieve better transport and shipping possible to protect the cellular therapy product integrity, throughout cooperation among facilities and considering suggestions, needs and expectation.
Results: Decisions led to:
a) use a computer software to avoid overlapping activities including shipping caused by TP complexity;
b) define and adopt a unique transport procedure liquid and cryopreserved HPC in the TP;
c) defined 5% as maximum vitality lose after transport;
d) define data returned to the PF regarding transport;
e) use of a unique courier for road transport.

From about a year we successfully fulfil what just said.
Although the main goals were shipping and transporting, we also obtained an easier way of doing it reducing expenses.
Conclusion: Following JACIE leads to organizational changes. Therefore PF political role has changed from just a service to a central coordinative role in planning TP activities. Slowly the PF has become a HPC "crossroad", playing a central role in defining methods to maintain the best cellular therapy product quality in transportation and shipment, throughout the all TP. Finally these changes can prevent the PF performance collapse caused by the increasing of activity.

P832

An approach for risk management in a cell therapy unit: identifying failure points of the quality control process

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Background and objectives: The aim of risk management is to identify every critical situation that could conceal a possible fault, active or not. A significant change in the rate of adverse events registered in our cell therapy unit led us to investigate potential causes, in order to highlight the risks associated with quality control of cell therapy products. In our approach, information collected by the quality management system and risk analysis technique have been combined to identify areas for improvements.
Design:
1) A retrospective study of adverse events recorded by personnel of cell therapy unit during 3 years.
2) We applied failure mode, effects and criticality analysis (FMCEA) to the quality control process.

Main outcome measures:
1) Categorisation of critical process stages, patent errors, and major failures from the adverse events record.
2) Gradation of failures modes according to their Risk Priority Numbers, which reflects occurrence, severity of the potential effect, and detection probability.

Results: Following the retrospective study of adverse events, among 7 different process stages, 3 are identified as critical: errors in registration and labelling of products and samples (7/7 patent errors, 1/7 led to a major failure); errors in evaluation of quality control results (6/7 patent errors, with no major issue); errors in validation and traceability prior to

the release of the product (7/11 patent errors, with 3 labelled as major failure).
Following the FMECA, among the 25 identified and analysed potential hazards, 2 have obtained a RPN score > 150: labelling of products and samples prior to analysis (RPN = 175; risk of serious erroneous validation of the product); registration of patient characteristics in the quality assurance software (RPN = 196; risk of repeated intrinsic errors due to the lack of integration of the software system).
Conclusion. Work is ongoing to modify the areas of quality control, identified to have the highest risk. This includes a redesign of the traceability form of the pre-analytical registration phase, and staff meetings about new quality assurance policies. To increase safety of the process and maintain the products' quality throughout the whole control steps, it is necessary to conduct critical evaluations focused on risks reduction.

P833
Quality management system: 3-years experience in a JACIE-accredited centre
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A Quality Management System (QMS) is a mechanism to ensure that procedures are being carried out in line with agreed standards and full participation by all members of staff. In a SCT programme, this assumes that the clinical, collection and laboratory units are all working together to achieve excellent communication, effective common working practices and increased guarantees for patient safety.
Department of Haematology and Oncology Charles University Hospital Pilsen includes 1 Clinical Unit (CU), 1 Apheresis Unit (AU) and 1 Cell Processing Laboratory (CPL). Our center started the preparative works to get JACIE accreditation with implementation of the QMS in the begining of 2007. A dedicated Quality Management Group (Medical Director, Deputy Medical Director, Head Nurse, Heads of CU, CPL, AU and Quality Manager) established the QMS (standard operating procedures, internal audits, adverse events detecting, specific training etc.) according to the JACIE standards. An on-site inspection visit was carried out by JACIE inspectors in the June 2008 and JACIE accreditation was awarded to our institution on October 2008. At present, after 3 years, Quality Management Group is verifying the functionality of the QMS and passes throug all documents to improve QMS for the future.
Now we focus on the results of 2 important parts of QMS – internal audits and adverse events reporting. Within the last 3 years 13 Quality Management Meetings were carried out at our institution and all key part of the QMS were discussed. During the course of internal audits 15 minor deficiencies were identified (6 in the 2008, 4 in the 2009 and 5 in the 2010 year), detected errors were not systemic. In the year 2008 we observed 51 adverese events, 20 (39%) in the CPL, 6 (12%) in the AU and 25 (49%) in the CU. In the year 2009 26 adverse events were detected, 12 (46%) in the CPL, 3 (12%) in the AU and 11 (42%) in the CU. 38 adverse events in the year 2010 were found, 12 (32%) in the CPL, 8 (22%) in the AU and 19 (50%) in the CU.
Conclusion: QMS is a means of rapidly identifying errors or accidents and resolving them so that the possibility of repetition is minimized. Comparing the first year QMS functioning at our department, significantly lower number of adverse events were reported in the next 2 years. We can conclude, that QMS system is now firmly established and leads to significant improvements in different aspects of our transplant programme.

Graft-versus-host disease – preclinical and animal models

P835
Contribution of sex-mismatched HY antigen expression and its cross-presentation in the induction of GvHD despite the absence of epitope spreading
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The current paradigm indicates that epitope spreading is critical for GVHD after MHC matched minor antigen (miHA) mismatched BMT. We tested relevance of epitope spreading by inducing GVHD in the presence of only a single immuno-dominant Y antigen disparity. We utilized female (F) male(M) MHC matched BMT with anti-H-Y monospecific, H-2(b)-restricted T cell receptor transgenic (TCR Tg) MataHari CD8+T cells . B6 [M and F] animals were lethally irradiated and transplanted with TCD BM and $1x10^6$ splenic CD8+T cells from [female] MataHari donors. While none of the syngeneic female animals developed GVHD, all of the allogeneic male animals showed severe clinical GVHD and died after BMT ($P<0.0001$, see Table). GVHD specific death was confirmed by target organ (GI tract and liver) histopathology on day +7 after BMT ($P<0.005$). We next titrated the dose of the MataHari T cells by several folds and mixed them with syngeneic T cells at varying ratios from male B6 donors. Significant GVHD mortality was observed in the MataHari group ratios ≥ 1: 10 demonstrating that the presence of sufficient precursor T cells against a single immuno-dominant miHA can induce significant GVHD even in the absence of epitope spreading. GVHD was also observed when two other anti-H-Y specific TCR Tg CD4+ (Marilyn and Rachel) were utilized ruling out strain dependent artifacts. Infusion of $1x10^5$ tetramer + HY-specific T cells harvested from male antigen primed WT female donors also induced GVHD in male recipients demonstrating that this was not an artifact of transgenic T cells. Mechanistic studies were performed to determine whether direct and/or cross-presentation (on host or donor derived APCs) of the immunodominant miHA is critical for GVHD. [F into M], [II-/-into M] and [Min to F] bone marrow chimeras generated, lethally re-irradiated and injected with TCD-BM and Marilyn CD4+ T cells. The [F into M], [II-/- into M] animals (express HY antigen on only non-hematopoietic target cells) died from severe GVHD while the [MintoF] animals (express HY antigen on only hematopoietic cells) showed only modest GVHD. Collectively our data (see Table) demonstrate that (a) in contrast to the current dogma, epitope spreading is not always required for GVHD (b) the immunodominant antigen expression on target tissues is critical and (c) this antigen can be efficiently cross-presented by either host or donor APCs.

GVHD across single immuno-dominant minor Y antigen

Donor T cells (all of B6[female] TCDBM)	Recipient	GVHD mortality * P<0.01
MataHari (CD8+ Tg, $1x10^6$)	B6 (female)	0%
MataHari (CD8+ Tg, $1x10^6$)	B6 (male)	100%
MataHari (CD8+ Tg, $0.1x10^6$)	B6 (male)	33%
Marilyn (CD4+ Tg, $1x10^6$)	B6 (male)	100%
Rachel (CD4+ Tg, $1x10^6$)	B6 (male)	100%
Marilyn (CD4+ Tg, $1x10^6$)	[B6[female] into B6[male]	100%
Marilyn (CD4+ Tg, $1x10^6$)	[B6(male) into B6[female]	25%
Marilyn (CD4+ Tg, $1x10^6$)	[II-/- into B6[male]	100%

P836

Vitamin A deficiency impairs gut-homing of donor T-cells and leads to prolonged survival in experimental GvHD

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Objective: Migration of donor T cells to target organs is a prerequisite for the development of GvHD in allogeneic bone marrow transplant (BMT) recipients. Migration to the intestine is achieved by specific expression of integrin α4β7 and chemokine receptor 9 (CCR9). Expression of these gut-specific homing receptors is supposed to be imprinted during naïve T cell activation in recipients' gut-associated lymphoid tissues (GALT) and the Vitamin A metabolite retinoic acid (RA) has been identified as the key factor in this process. Here we address the role of Vitamin A deficiency in BMT recipients for donor T cell migration in the course of experimental GvHD.
Methods: Vitamin A deficient (VAD) BALB/c mice were prepared by feeding them a VAD diet over 6 weeks from gestational day 14. Experiments were performed in a C57BL/6 into BALB/c mouse model of acute GvHD. VAD and control mice were used as BMT recipients and wildtype, CCR9-/- and β7-/- C57BL/6 mice were used as donors. GvHD severity was monitored by survival, clinical score and histological analysis. Trafficking of donor T cells was assessed at different time points after BMT.
Results: Expression of α4β7 and CCR9 in GALT was inhibited in VAD mice when compared to mice fed with standard diet at day 3 after BMT. Competitive in vivo homing assays showed that allogeneic T cells primed in VAD mice do not home to the intestine as efficient as allogeneic T cells primed in normal fed mice. Vitamin A-deficiency in recipients of an allogeneic stem cell graft prolongs survival from GvHD similar to control recipients which received β7-/- donor T cells. Of note, control recipients which received CCR9-/- T cells did not have prolonged survival as compared to wildtype grafts.
Conclusion: The results of this study show that Vitamin A in BMT recipients is a prerequisite for the induction of α4β7 and CCR9 on allogeneic donor T cells and migration to the intestine in an experimental GvHD model. The prolonged survival of VAD recipients is most probably due to a lack of expression of α4β7 and not CCR9 on donor T cells.

P837

Antibody-mediated prevention of graft-versus-host disease and preservation of graft-versus-leukaemia

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Graft versus Host Disease (GVHD) is the major limitation of allogeneic hematopoietic stem cell transplantation (HSCT). Pre-transplant conditioning activates the residual host immune system driving interactions between host dendritic cells (DC) and donor T lymphocytes resulting in GVHD. Current therapies for GVHD focus mainly on T-cells and whilst effective it reduces patient immunocompetence, compromising graft versus leukaemia (GVL), engraftment and protection from infection. The pivotal role of DC in GVHD suggests that their depletion may control GVHD by preventing T cells from being sensitised to host antigens without impairing immunity. Using a rabbit polyclonal IgG anti-mouse CD83 (RAM83) antibody (Ab) we found that RAM83-treatment of LPS activated splenocytes in a mixed lymphocyte reaction (MLR) reduces the proportion of proliferating CD4+ T cells but not CD8+ T cells. A single dose of RAM83 on day-1 of transplant delays GVHD onset in a myeloablative conditioned MHC-mismatched model of murine HSCT (p=0.001). Similarly, repeat dosing of RAM83 conferred a survival advantage to minor histocompatibility (miHA) mismatch HSCT recipients, a clinically relevant sibling transplant model (Fig 1). Analysis of the effect of RAM83 on engraftment and immunity post transplant demonstrated unchanged proportions of donor T, NK, myeloid cells but a significant reduction in donor CD19+ B cells early post HSCT compared to control IgG or KT3 (T cell depleting Ab)-treated HSCT recipients. Donor cells from RAM83-treated HSCT recipients have attenuated antigen presentation capacity but not T cell function as assessed by MLR. We then conducted a timed sacrifice experiment where conditioned BALB/c mice received an allogeneic transplant with or without the BALB/c leukemia, BCL1, and RAM83 or control IgG. RAM83 did not compromise GVL. Cohorts receiving RAM83 tended to have slightly larger spleens and livers than cohorts receiving control IgG but the latter had higher GVHD scores and more donor NK cells (Fig 2). We extended these studies to show that allogeneic HSCT recipients with BCL1 receiving RAM83 have an enhanced GVL effect as measured by spleen size and the number of BCL1 cells, compared to cohorts receiving KT3, or daily doses of cyclosporine. Our results suggest that the CD83 antibody delays or prevents GVHD onset by RAM83-mediated reduction in CD4 T cell proliferation but does not impact CD8 T cell function ensuring maintenance of anti-leukemic activity.

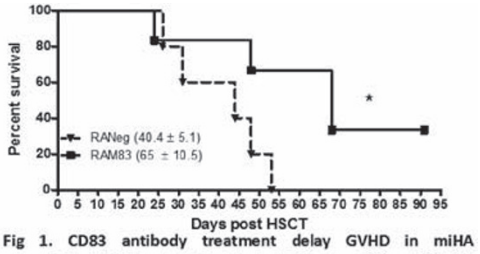

Fig 1. CD83 antibody treatment delay GVHD in miHA mismatched HSCT recipients. Myeloablative conditioned BALB.B mice received 10^7 BM & 10^7 splenocytes from UBI-GFP/BL6 mice. GVHD prophylaxis was 1 mg/mouse RANeg on d-1 & d14 or d14 or 1 mg/mouse RAM83 on d-1 & d14 or d14. Mice were monitored for GVHD onset. Day of death (mean±sem)*p=0.08

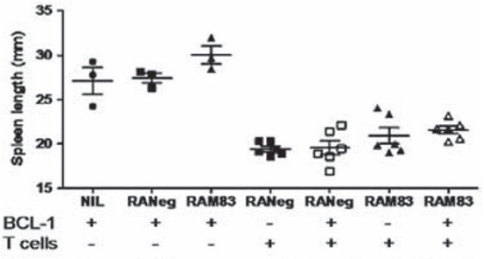

Fig 2. CD83 antibody treatment of MHC-mismatched HSCT recipients does not prevent GVL. Myeloablative conditioned Balb/c mice received a combination of 10^7 BM with ± $5x10^6$ splenocytes from UBI-GFP/BL6 mice ± 10^4 BCL-1 leukaemia cells. GVHD Prophylaxis was Nil, 1mg/mouse RANeg or RAM83 on d-1. Mice were sacrifice on day 14 and assessed for GVHD and GVL indices. Spleen measurement indicates growth of leukaemia (n=6, bar indicate mean ± sem).

P838

CD83-antibody therapy to prevent GvHD: impact on B cell reconstitution

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Humoral and cell-mediated immunity are impaired in conventionally immunosuppressed allogeneic hematopoietic stem cell transplant (HSCT) recipients, resulting in infection and

relapse. We have shown previously that rabbit polyclonal anti-mouse CD83 (RAM83) antibody (Ab) delays Graft versus Host Disease (GVHD) in murine allogeneic HSCT recipients and preserves T cell function. CD83 is expressed on activated dendritic cells (DC), monocytes, B cells and CD4+ blasting T cells. Pre-HSCT conditioning damages the gut releasing LPS, which leads to CD83 expression on activated DC and B cells that can be further upregulated by allogeneic stimulation. Therefore, both host and donor B cells as well as DC could be targets of RAM83, and depletion of donor B cells may result in impaired humoral immunity post HSCT. Timed sacrifice experiments in a full MHC mismatch HSCT model compared the effect of RAM83 and the T cell depleting Ab, KT3 on engraftment. RAM83-treated recipients had delayed B cell reconstitution (d7, 14, 21 post-HSCT). However, in a MHC matched, minor histocompatibility antigen mismatched HSCT model, RAM83 and nil treated recipients had similar proportions of donor B cells at sacrifice for severe GVHD (mean day of death 65±10.5, 40.4±5.1, respectively), suggesting normalisation of B cell reconstitution over time. In syngeneic HSCT, without GVHD, BM and splenic B cell numbers were the same in nil and RAM83 treated mice, in contrast to the allogeneic HSCT setting. However, a significant reduction in CD83+ immature B cell subsets and mature B cells at d7 in RAM83-treated HSCT recipients was seen ($P<0.04$, $n=6$). In vitro experiments confirmed that RAM83 depletes CD83+ immature B cells (pre-pro B, pro B, pre B immature B cells). Interestingly, we also found that RAM83 enhanced antigen specific IgG and IgM Ab responses in LPS-treated or naive untransplanted mice. These disparate findings on the effects of RAM83 treatment on the proportion of B cell subsets in syngeneic and allogeneic HSCT recipients and on antigen specific immune responses suggests that anti-CD83 antibody therapy to control GVHD in allogeneic HSCT may affect humoral immunity in the short term post HSCT.

P839

Diagnostic performance of soluble cytokeratin 18 for the early detection of gastrointestinal graft-versus-host disease

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Cytokeratin-18 (CK18) is an intermediate filament in epithelial cells, expressed in colon, placenta, liver and lymph nodes. CK18 is cleaved by caspases during apoptosis of epithelial cells and fragments thereof are released into the serum. Since apoptosis is one of the hallmarks of graft-versus-host disease (GvHD), CK18 can potentially be used as a biomarker for tissue damage during apoptosis. GvHD is a frequent and life threatening complication after allogeneic hematopoietic cell transplantation (HCT). Histological diagnosis of GvHD is not always feasible, since the affected tissues are not reachable due to different clinical conditions. Therefore a serum biomarker in this setting is a valuable resource for GvHD presumptive diagnosis. We analyze the diagnostic usefulness of soluble CK18 in patients undergoing allogeneic HCT for GvHD assessment. Serum samples were regularly obtained from 38 patients before and after transplant for a period of 150 days. Patient serum levels pre-transplant showed no statistical significant differences compared with CK18 levels in healthy individuals. In 20% of patients without GvHD increments in serum CK18 were detected, while almost all patients (92%) with gastrointestinal GvHD (GI-GvHD) showed increments of more than 50% with respect to the pre-transplant sample. Sensitivity of CK18 for GI-GvHD detection was 0.92, while a negative predictive value of 0.91. In patients with only skin GvHD, sensitivity was 0.60 with a negative predictive value of 0.71. To analyze the general performance of the test, receiver-operator curves (ROC) were performed. The ROC curves showed an area under the curve (AUC) close to 0.90 and this was statistically significant, for sCK18 tested in patients with GI-GvHD (AUC 0.822, 95% CI, 0.659 to 0.985; $p<0.01$). In line with the sensitivity, specificity and predictive values found in the group of patients with skin GvHD, ROC curve for sCK18 showed a poor performance in this group with AUC of 0.618 (95% CI, 0.389 to 0.847) and no statistical significance. Interestingly CK18 levels were raised before clinical GvHD manifestations in 10 out of 12 patients with GI-GvHD (83%). Our findings suggest that serum CK18 is an early and sensitive biomarker for GI-GvHD detection. It can be used as a screening tool in patients undergoing allogeneic HCT, especially when an endoscopic biopsy is clinically indicated but not feasible to perform.

P840

Rituximab induced T-cell inactivation as a potential therapy for graft-versus-hHost disease

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Allogeneic stem cell transplantation (Allo SCT) remains the treatment of choice in many hematological malignancies, providing essential graft versus tumor effect. However, this potential advantage is often offset by a high mortality rate caused by graft vs host disease (GvHD). Recent data suggest rituximab may control chronic GvHD by depleting B cells that participate in this process. The current in vitro study investigates whether this beneficial effect is also mediated through anti T cell activity.
Methods: CD25 depleted T cells were stimulated with allogeneic Dendritic cells (DCs) in the absence or presence of 2 mg/ml rituximab, simulating the allogeneic stem cell transplant setting in which donor T cells are activated by host DCs. T cell activation capacity was assessed by measuring the expression of activation markers, inflammatory cytokine production and proliferation competence.
Results: T cell responsiveness to allogeneic DCs was markedly attenuated in the presence of rituximab, reflected by (a) a reduced expression of CD25, GITR and CTLA activation markers (b) a reduced inflammatory cytokine production (c) decreased T cell proliferative capacity (Table 1). T cell proliferation (O.D) in response to other stimuli, including CMV peptide and anti CD3/CD28 were also markedly reduced (1.56 to 0.57 and 0.7 to 0.1, respectyively). Interestingly, rituximab-induced T cell inactivation was independent of the existence of B cells in the culture. Stimulation of B cell depleted T cells (CD3+CD25-CD19-) with allogeneic DCs in the presence of rituximab resulted in a significantly diminished T cell response. Rituximab was demonstrated to directly bind to CD3+CD20+, pro-inflamatory T cells, resulting in their disappearance.
Conclusion: Rituximab results in dose dependent T cell inactivation. This effect appears to be non-B cell dependent, being obtained in the absence of B cells in the culture, and might be mediated through rituximab binding to a CD3+CD20+ T cells. Rituximab-induced T cell inactivation, most prominent using high concentrations, might be valuable in the Allo SCT, providing control of both acute and chronic GvHD.

Table 1. T cell Activation profile

		Without rituximab	With rituximab
*Activation marker (%)	CD25	27	9
	GITR	15.6	4.7
*Cytokines expression (%)	IL-2	22	2
	IL12	16	4
	IFN-gamma	21	1.8
T cells proliferation (O.D.)	DC stimulation	1.528	0.580

*Gated out of lymphocytes

P841

Combination therapy of allogeneic stem cell transplantation, donor lymphocyte infusion and dendritic cell vaccination induces superior graft-versus-myeloma responses without acute graft-versus-host disease

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Allogeneic stem cell transplantation (alloSCT) followed by donor lymphocyte infusion (DLI) can induce graft-versus-myeloma (GVM) responses and long-term complete remission in patients with multiple myeloma (MM). However, since still about two-third of the patients relapse, this therapy needs further improvement. Since minor histocompatibility antigen (MiHA)-presenting dendritic cells (DCs) are crucial for the induction of alloreactive responses, we tested the effectiveness of DC-vaccines in a preclinical mouse model for MM. We show that DLI significantly improved alloSCT which was paralleled by the presence of residual recipient-derived DC precursors in lymphoid organs. Importantly, a single vaccination with MiHA-loaded DCs, but not unloaded donor or recipient DC, further improved the immunological and clinical response, without inducing significant acute graft-versus-host disease. Moreover, we observed that MM growth in the bone marrow induced the accumulation of effector CD8+ T cells expressing the inflammatory chemokine receptor CXCR6. This recruitment was correlated with a more than 10-fold increase in the expression of IFN-γ and inflammatory chemokines, including CXCL9, CXCL10 and CXCL16. Remarkably, inflammatory responses in MM-bearing spleens were completely lacking. Over time, this was correlated with a shift of the MM growth from the bone marrow to the spleen. Our data demonstrate that DC-vaccination in combination with alloSCT and DLI induces superior GVM responses.

P842

Proper adhesion molecule expression on non-haematopoietic tissues is a requirement for GvHD reactivity by alloreactive effector T-cells

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Based on the clinical observation that high frequencies of circulating T cells specific for mHags with ubiquitous expression on both hematopoietic and non-hematopoietic cells can be detected in patients showing strong GvL with no or limited coinciding GvHD, we hypothesized that non-hematopoietic tissues are relatively unsusceptible to the cytotoxic effect of mHag-specific T cells under normal, non-inflammatory conditions. To test this hypothesis, we investigated the functional reactivity of allo-reactive T cells specific for ubiquitously expressed mHags against skin-derived primary human fibroblasts as non-hematopoietic target cells. In standard 4 hours cytotoxicity assays only 10-20% CTL-induced lysis of fibroblasts was found. Artificially increasing the amount of peptide-MHC complexes by exogenous peptide loading or increment of the amount of effector cells did not improve the susceptibility of the fibroblasts, indicating that other factors besides from the number of TCR-peptide/MHC interactions play a crucial role in target cell susceptibility. Interestingly, we observed that upon fibroblast encounter a portion of the effector T cells was activated and released their cytotoxic granules, but this did, however, not result in proper target cell lysis. Fluorescent confocal microscopy analysis of the interactions between effector cells and fibroblasts revealed the inability of both cell types to form high avidity interactions under steady state conditions, thereby explaining the ineffectiveness of the observed T cell degranulation. Next, we demonstrated that prolonged co-culture significantly increased the effectivity of T cell mediated fibroblasts lysis, coincided by the local production of IFNg by the activated T cells. This IFNg production caused profound induction of CD54 expression and upregulation of HLA class I on the fibroblasts. Indeed, IFNg pretreatment of fibroblasts strongly increased their capacity to form high avidity interactions with the effector T cells, resulting in increased susceptibility to T cell attack. Via retroviral transduction we demonstrated that CD54 was the key molecule in this IFNg-mediated immuno-sensitization of the fibroblasts. In conclusion, we demonstrate that the occurrence of target cell damage in the effector phase of GvHD is not only determined by the tissue expression profile of the mHags targeted in the immune response, but is dependent on the induction of adhesion molecule expression on the GvHD target tissues.

P843

The presentation of an exogenous minor HA by host but not donor APC initiates GvHD; however, dendritic cells are redundant

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Allogeneic bone marrow transplantation (BMT) is the preferred curative therapy in the majority of haematological malignancies and severe immunodeficiencies. Despite improvements in BMT, acute graft-versus-host disease (GVHD) remains a significant problem after transplantation, and it is still a major cause of post-transplant mortality. The relative ability of a minor histocompatibility antigen (mHA) to initiate GVHD when presented within class II by host versus donor APC subsets is unknown. This is critical for the development of clinical therapies based on antigen presentation to control deleterious alloreactive responses. We developed a BMT system whereby GVHD directed against a processed I-Ed peptide within I-Ab on APC can be initiated using the transgenic TEa T cell. Recipients were lethally irradiated wild-type (wt) B6D2F1 (H-2b/d) mice or F1 offspring from B6.MHC class II-/- and DBA/2 parents (host APC express and present the mHA or express the mHA but cannot present it respectively). Donor grafts were bone marrow from wt B6 or B6.MHC class II-/- mice (donor APC can or cannot present the mHA respectively) and sort-purified luciferase expressing TEa T cells. The transplantation of 100,000 TEa T cells resulted in GVHD mortality with a median of 8.0 days when mHA was presented by host APC, regardless of the ability of donor APC to additionally present the mHA. In contrast the presentation of the mHA by donor APC alone failed to induce GVHD mortality by day 50 ($P<0.0001$). T cell titration studies confirmed that host APC were 100-1000 times more potent than donor APC in inducing acute GVHD. In vivo bioluminescence revealed antigen presentation occurred initially in the mesenteric LN with subsequent T cell infiltration into the gut. When using CD11c.DOG F1 recipients (express both DTR and ovalbumin off of the CD11c promoter), the specific deletion of host DC prevented the priming of ova-specific OT-II cells but surprisingly enhanced priming of alloantigen-specific TEa T cells. GVHD to mHA presented in MHC class II is initiated by host APC however DC are not required. Therapeutic strategies to prevent GVHD should target antigen presentation rather than DC depletion.

P844

Modulation of alloantigen-induced immune responses by stromal cells isolated from placental tissue

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Bone marrow-derived mesenchymal stromal cells (MSC) have immunosuppressive properties in allogeneic settings and have been used to treat steroid-refractory acute graft-versus-host disease (GVHD) in stem cell transplant patients. Fibroblast-

like stromal cells with multipotent differentiation capacities can also be isolated from term placental tissue, which share many features with MSC in terms of differentiation potential and surface marker expression. The aim of this study was to examine the immunoregulatory effect of placental stromal cells in allogeneic settings. We have isolated stromal cells from term placentas, umbilical cords and amniotic membranes from three donors and compared their immunoregulatory capacity in mixed lymphocyte reactions (MLR). Peripheral blood mononuclear cells (PBMC) from at least 16 donors were stimulated with allogeneic PBMC, in the presence or absence of amnion stromal cells (SC), cord-SC or placenta-SC (SC/PBMC ratio 1:10). Amnion-SC and cord-SC significantly suppressed alloantigen-induced proliferation (median 48% and 28% suppression, $p<0.001$ and $p=0.04$, respectively), whereas placenta-SC had no significant effect on proliferation. Cytokine levels in supernatants from these cultures were analysed by ELISA. Addition of cord-SC and placenta-SC significantly increased the secretion of IL-17 (mean 204 pg/ml ($p=0.02$) and 306 pg/ml ($p=0.03$), respectively) compared to control cultures (mean 121 pg/ml), whereas amnion-SC instead suppressed IL-17 production from alloantigen-stimulated PBMC (mean 54 pg/ml, $p=0.03$). Amnion-SC and cord-SC increased IL-10 secretion ($p=0.007$ and $p<0.001$, respectively) compared to control MLR. IFN γ production was not affected by the presence of any of the stromal cells tested. To conclude, stromal cells from amniotic membrane have great immunosuppressive capacity without inducing harmful inflammatory cytokines, such as IL-17, and could potentially be used for treating acute GVHD.

P845

Reconstitution of human dendritic cell populations in humanized mice

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After allogeneic hematopoietic stem cell transplantation, persisting host dendritic cells (DC) play a critical role for initiating acute graft-versus-host disease (GvHD). They are therefore interesting targets for strategies to prevent GvHD. Many potential DC-modifying agents (e.g. antibodies) are species-specific and preclinical models are lacking. We therefore aimed on reconstituting human DC in a humanized mouse model.

For this purpose, 4 to 9 week old NSG-mice were sub-lethally irradiated (1.5 Gy) and $1x10^6$ to $5x10^6$ human peripheral blood stem cells (PBSC) were injected intravenously. Mice were divided in 5 or more cohorts of 3 to 4 animals. After 6 weeks, bone marrow (BM), spleen, and peripheral blood (PB) of the first cohort were analyzed by flow cytometry. Subsequent cohorts were examined accordingly, allowing us to follow hematopoietic reconstitution for at least 30 weeks. Five experiments with PBSC of 3 different donors were performed. In week 6, all of the transplanted mice engrafted with human CD45-positive cells in BM (mean 37.07%) and PB (mean 4.69%). We found CD19-positive B cells, NK cells, and CD14-positive monocytes as well as myeloid DC (HLA-DR+/CD11c+) in BM and spleen. In contrast, T cells were not present before week 24. Among a population of CD3-negative CD4-positive cells, present in the BM as early as week 6, we detected CD123-positive plasmacytoid DC. First data suggest that the administration of Fc-FLT3L (20 µg) promotes early reconstitution of monocytes and myeloid DC. In contrast, Fc-IL7 and low-dose mitoxantrone had no impact.

We stained skin-biopsies for the presence of human Langerhans cells (LC) as surrogates for the reconstitution of tissue-resident DC. In some models, the first human CD207/CD1a-positive LC were detected in the dermis as early as 4 weeks after transplantation, populated the epidermis beyond week 6, and even became confluent beyond week 15. LC-engraftment was not consistent in all experiments and conditions supporting LC-reconstitution need to be further defined.

In summary, we confirmed previous data that different human DC populations engraft in PBSC-transplanted NSG-mice. Reconstitution of myeloid DC and monocytes seems to be promoted by Fc-FLT3L. For the first time, we demonstrate the establishment of human LC in murine epidermis. PBSC-transplantation of NSG-mice could help to define factors promoting DC-reconstitution. The humanized model might also be valid to test DC-targeting strategies in vivo.

P846

Immuneregulation of graft-versus-host disease in bone marrow transplanted mice using activated mesenchymal cells and kynurenine

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Objectives: Graft-versus-host disease (GVHD) caused by activated immune cells remains a major problem in allogeneic hematopoietic stem cell transplantation (HSCT).

Mesenchymal stem cells (MSC) are increasingly used to treat refractory GVHD. However, human clinical trails show inconclusive results.

Stimulation of MSCs with interferon γ has been shown to induce immunosuppressant effect on T cell proliferation, by the activation of the Kynurenine pathway. In this pathway, Indoleamine 2,3-dioxygenase (IDO), an intracellular enzyme, catalyses the catabolism of Tryptophan to Kynurenine metabolites. These metabolites have the ability to suppress T cell activation in the absence of MSCs. However, their influence on GVHD prevention has not yet been investigated.

In this study we investigated the influence of interferon γ stimulated/non-stimulated MSCs or Kynurenine alone on T cells proliferation and cytokine secretion, in vitro. In addition we examined the use of Kynurenine for GVHD prevention in vivo.

Methods: For the in vitro studies, we have obtained mouse splenocytes, separated T cells and activated them using concanavalin A, CD3 and CD28 antibodies or allogeneic splenocytes in the presence of stimulated MSCs or Kynurenine. Proliferation was evaluated by thymidin uptake and the levels of cytokines in the media were measured using mouse cytokine array or ELISA.

For the in vivo study, mice were transplanted with bone marrow added with spleen cells from semialogeneic donors. Soluble Kynurenine was administrated intravenous for two weeks starting at the day of transplantation. The clinical condition and survival of the mice were monitors for one month after transplantation.

Results: We demonstrate that interferon γ Stimulated MSCs or Kynurenine alone have the ability to decrease allogeneic activated T cells proliferation. Moreover, Kynurenine decreases the secretion of inflammatory cytokines, such as IFN γ and TNF α and Th2 cytokines such as IL5 and IL4 while increasing the secretion of the regulatory cytokine IL10 from activated T cells, in vitro.

In vivo we have seen that Kynurenine administration leads to significant decrease in GVHD score and an improvement in mice survival.

Conclusion: These results suggest that Kynurenine administration may have therapeutic potential for preventing GVHD in the clinic.

P847

Optimization of NK cell therapy in the GvHD mouse model

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Natural killer (NK) cells are a promising tool for cell therapy because of their capacity to lyse tumor cells without prior activation and their influence on the innate as well as the adaptive immunity. It is known that NK cells are a heterogeneous population and can be divided into functionally distinct NK cell subpopulations. Murine NK cells can be separated along their expression of CD27 and CD11b. We have further demonstrated the expansion of CD117 (c-kit) expressing NK cells with immunosuppressive functions during tumor progression. Clinical studies exploiting the impact of NK cells during hematopoietic stem cell transplantation (HSCT) have provided promising results. However, the functional relevance of the distinct NK subsets in graft-versus-host-disease (GVHD) has not been investigated in detail so far.
We have established different protocols for isolation and expansion of murine NK cell subpopulations. These NK subsets were further analyzed in vitro and in vivo in an allogeneic murine GVHD model. The NK preparations provide differences in their genomic, phenotypic and functional properties. Data clearly demonstrate that CD11b+ NK cells express multiple genes of cytotoxic pathways and develop the highest tumorlytic capacity. We observed up to 60% tumor lysis by CD27- CD11b+ NK cells compared to 40-45% by CD27+ CD11b+, about 25% by CD27+ CD11b- and 10% by c-kit+ CD11b- NK cells at an effector-target ratio of 5:1. Interestingly, CD27+ NK cells provided the highest IFN-g production upon incubation with tumor cells and/or IL-2. We further analyzed the polarization of different NK cell subpopulations and their functional capacities upon stimulation and expansion in different cytokines such as IL-2, IL-18 and IL-15. Cultivation of NK preparations in IL-2 enhanced their cytotoxic capacity and IFN-g secretion. Addition of IL-15 led to a higher proliferation rate. Interestingly, IL-18 stimulated NK cells up-regulated the expression of c-kit, a phenotype associated with immunosuppressive and regulatory functions.
Finally, we investigated the role of distinct NK cell preparations or subpopulations in the development of GVHD in a fully MHC mismatched HSCT mouse model. In summary, our comparative study outlines that NK cells need specific stimuli to provide GVHD protection. Especially one subpopulation of NK cells stimulated with IL-2 fulfills the criteria to attenuate GVHD.

P848

Clinical, endoscopic and molecular characterization of murine GvHD colitis

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Hematopoietic stem cell transplantation (HSCT) often represents the only curative option for severe forms of haematological malignancies. 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 frequently suffer from severe intestinal inflammation. Most therapeutic agents designed to reduce gastrointestinal GVHD induce systemic immunosuppression that often goes along with an unwanted reduction of the graft-versus- leukaemia (GVL) effect and negative impact on disease free survival. An improved understanding of the exact immunopathogenesis of intestinal GVHD is needed in order to develop innovative therapeutic strategies which could be able to target the harmful GVHD reaction without affecting GVL mediated benefits.
In our study, GVHD colitis was characterized in a murine model of allogeneic mismatch BMT following myeloablative irradiation with an allogeneic T cell transfer. In vivo endoscopic imaging enabled us to monitore the course of GVHD colitis over time.
To establish an endoscopic score for murine GVHD colitis, we first performed comparative analysis of GVHD colitis and TNBS colitis, a common model for inflammatory bowel disease.
Endoscopic imaging of GVHD colitis at week 3 after BMT and T cell transfer revealed strong inflammation of the distal colon associated with clinical symptoms (diarrhea, weight loss) and an increased infiltration of immune cells within the intestinal mucosa (HE staining). At the same time point, control mice (BMT w/o T cell transfer) showed significant lower endoscopic colitis scores and did not develop clinical symptoms. In our model, GVHD mice did not recover from colitis even 35 days after GVHD induction, indicating a chronic course of colonic inflammation. During GVHD colitis, we observed high T cell infiltrates in lymphoid and non-lymphoid organs including the colon as well as loss of Treg and a peripheral loss of NK cells that has not been observed in the BMT control group. High colonic expression levels of the transcription factor Tbet and the pro-inflammatory cytokine IFN-g in GVHD mice were detected by quantitative PCR and indicated a TH1 polarization of infiltrated immune cells.
In conclusion, 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.

P849

Increased miRNA17 and miRNA20a expression in patients with development of acute graft-versus-host disease after allogeneic stem cell transplantation

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Objectives: MicroRNAs (miRNAs) are small non-coding nucleic acids which are well known to have a regulatory function for many gene expressions. It is described that they have a potential role in T-cell activation of immune mediated diseases. Some miRNAs are also known to be up-regulated in placentas of pregnant women during onset of preeclampsia. As recently published miRNA17 and miRNA20a were highly moderated in patients with multiple sclerosis. It seems that their dysregulation are essential for the induction of allogeneic immune tolerance in the transplant setting and during pregnancy. It is well known, that immune tolerance plays a crucial role in long-term allogeneic graft function, and the development and control of acute graft-versus-host disease (aGvHD) after allogeneic stem cell transplantation (aSCT) in patients with haematological diseases. This prompted us to analyse miRNA17 and miRNA20a in patients after allogeneic stem cell transplantation with aGvHD.
Methods: We analysed the expression of miRNA17 and miRNA20a using a realtime RT-PCR in mononuclear cells of at all 27 blood samples. 10 patients (median age 54, range 18- 69 years) with aGvHD grade II-IV after aSCT were compared to healthy volunteers (n=5), pregnant women (n=5), and patients with multiple sclerosis (n=7). GAPDH was used as endogenous reference. Patients were transplanted because of the following haematological diseases: Myelodysplastic syndrome RAEB II (n=3), acute leukaemia (n=6), and non-Hodgkins lymphoma (n=1). 7 patients were transplanted with a matched unrelated donor (MUD), 2 patients with identical sibling donor, and 1 patient with a haploidentical family donor. 4 patients developed aGvHD grade II, and 6 patients grade III–IV.
Results: In healthy volunteers a median of miRNA/GAPDH expression of 0.93% for miRNA17 and 0.06% for miRNA20a was measured. Pregnant women showed a comparable expression. In contrast to that, patients with multiple sclerosis

had a 4-fold higher expression for miRNA17 and a 2-fold higher expression for miRNA20a. Also in all analysed patients with aGvHD after aSCT a 3-fold higher expression was measured for miRNA17 (p=0.012) and miRNA20a (p=0.003). All tests were performed in duplicates.
Conclusion: These data suggest that miRNA17 and miRNA20a expression might be involved in the development of allogeneic immune tolerance. Due to the small number of patients, further analyses are needed to confirm these results.

P850
Co-stimulation blockade to modulate allogeneic T-cell responses
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Full T cell stimulation requires a coordinate cascade of signals provided by antigen-presenting cells (APC), including (1) T cell receptor binding to the MHC/antigen complex, (2) co-stimulation by interaction of CD80/86 on APC with CD28 on T cells and (3) cytokine signals. Previous reports suggest that antigen stimulation of T cells in the absence of co-stimulation induces sustained non-responsiveness in T cells specifically towards the respective antigen, while preserving T cell responsiveness to others. This state is compatible with antigen-specific tolerance. To achieve allo-antigen-specific tolerance is a major goal in hematopoietic stem cell transplantation (HSCT), as it will allow T cells to mount protective responses against infectious challenges while not aggravating graft-versus-host disease (GvHD).
In this study we present our continuing effort to explore, whether co-stimulation blockade would induce allo-specific non-responsiveness in T cells. In a murine model of using C57BL/6 spleen derived dendritic cells (DCs, CD11c+) as stimulators and Balb/c derived T cells as responders (major mismatch), we investigated the effect of co-stimulation blockade through CTLA4-Ig, using the native CTLA4-Ig fusion protein and abatacept, a pharmacologically prepared CTLA4-Ig fusion protein with a mutation in the Fc portion of IgG1. We found that both types of CTLA4-Ig fusion proteins dampened allogeneic T cell responses when present during T cell/DC co-culture. This dampening effect, however, was not due to CTLA4-Ig mediated induction of a regulatory DC phenotype, nor did CTLA4-Ig per se affect the proliferative capacity of isolated T cells, but CTLA4-Ig appeared to directly interfere with the DC/T cell crosstalk. The inhibitory effect was more pronounced in the CD4+ than in the CD8+ population. Moreover, CD4+ T cells when recovered from DC/T cell co-cultures performed in the presence of CTLA4-Ig exhibited a pronounced CD25high phenotype and displayed high levels of expression of CD62L and FoxP3, consistent with a regulatory phenotype. This regulatory phenotype appeared to be stable as it was maintained even after restimulation. In summary, costimulation blockade by CTLA4-Ig affected allo-antigen stimulated T cell responses quantitatively and qualitatively, by dampening the proliferative response and by inducing a T cell regulatory phenotype, respectively. Thus, CTLA4-Ig may be useful in the generation of allo-specific tolerance in HSCT.

P851
Cord blood transplantation: cord blood T-cell feature, IL-7 dependance
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Donor-derived CD4+ naïve T cells are the most important subset in triggering acute graft versus host disease (aGVHD) in HLA matched alllogeneic hematopoietic stem cell transplantation. However, despite a high proportion of CD4+ naïve T cell (near 90%), patients receiving allo-hematopoietic stem cell from unrelated HLA-mismatched cord blood (CB) had low aGVHD rate. In attempt to investigate this difference, we conducted in vitro study comparing cord blood CD4+ naïve T cells to adult CD4+ naïve T cells.
Materiel and methods: Cord blood CD4+ cells and adult blood CD4+ T cells were isolated from CBMC and PBMC using a FACS sorter. The main characteristic of the sort was that sorting cells were all alive (we used a vital dye). CD4+ CCR7+ CD45RA+ and CD25- T cells were all alive after the sort and were cultured with RPMI after a CFSE labelling. We compare cord blood naïve T cells and adult blood naïve T cell with or without allogenic dendritic cell stimulation (MLRDC: mixed lymphocytes culture with dendritic cells). With 10 cord blood naïve T cell cultures, we observed a spontaneous apoptosis of all the cord blood T cells within 6 days (less than 10% of viable T cell). The viability of adult T cells, in the same culture conditions, were preserved (near 90%). We conclude that naïve cord blood T cell, compared with adult T cells, were highly sensitive to survival factors.
In MLRDC condition, we observed a highly proliferation capacity of cord blood T cell, comparable to adult T cells as assessed by CFSE. A T cell stimulation and a T cell receptor engagement are able to protect the naïve cord blood T cell from apoptosis. IL-7 is an interleukin commonly knew as a T cell survival factor. At a 10ng/µL concentration, we observed a complete restoration of naïve cord blood viability: after 5 days of culture, 90% of T cell are alive. The IL-2 and INF-g assessment after MRLDC with cord blood T cell and adult T cell found a deep difference between the 2 populations: cord blood T cell are enable to produce Th1 cytokines; adjunction of IL-7 do not modify this observations.
Conclusions: Naïve cord blood T cells are highly sensitive to apoptosis and seem to be less functional than adult CD4+ naïve T cells and require cytokines to survive. Without stimulation or survival factor, all of them present apoptosis symptoms within 6 days. To protect the T cell from the death, they require of survival factor as IL-7.

Lymphoma

P852
Allogeneic stem cell transplantation for Hodgkin's disease: a retrospective analysis of data from the German stem cell transplantation registry (DRST) and GCTSG
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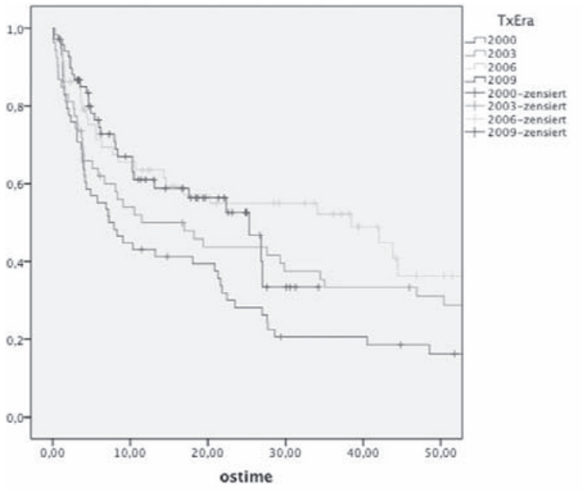
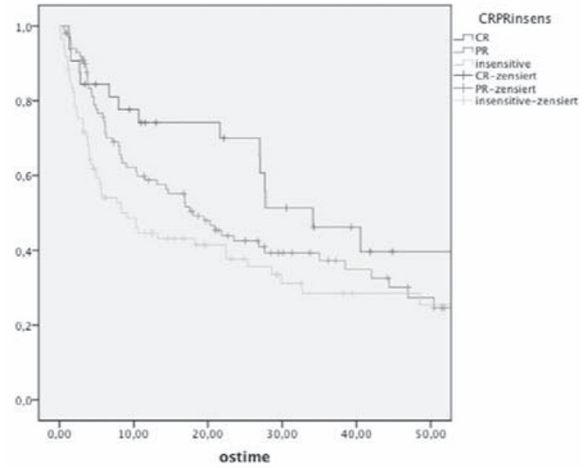
High dose chemotherapy with autologous stem cell transplantation is the standard of care for relapsed Hodgkin's disease with high remission rates. However a small proportion of these patients relapse and have been offered allogeneic transplantation, in particular when a matched sibling donor was available. However less information is available on results with unrelated donors and on potential factors influencing outcome. We performed a retrospective analysis of all allogenic stem transplants for Hodgkin's disease registered in the DRST and made a survey among the centers of the GCTSG to cross-check and update the information. Kaplan-Meier-analyses and log rank-tests were used for survival analyses. A total of 245 patients receiving an allograft from a matched related (n=110) or unrelated (n=135) donor between 1995 and 2009. 165 patients (67%) had received a prior autologous transplantation a median of 11.6 months before allografting. Disease status before allotransplant was CR in 13.5%, PR in

25.3%, sensititve relapse in 13.9% and progressive or resistant disease in 33.9% The median age at transplant was 31 yrs with a range of 11 to 64 yrs. The median follow up for surviving patients was 21 months. Median overall survival for all patients was 16.9 months. There was a strong correlation with the time of transplantation: median OS was 7.2 mo for patients transplanted up to 2000, 9.1 mo between 2001 and 2003, 38.5 between 2004 and 2006 and 25.3mo between 2007 and 2009. Fig. 1 shows overall survival in months depending in transplant era. There was no significant effect on survival for donor type, prior autograft or age (by decades). There was a clear trend that better response before allograft was associated with better surival with a median of 28 mo for patients in CR vs 8.4 mo for refractory disease, however this was not statistically significant (Fig. 2). In summary these data clearly show an improvement of transplantation results over the last years and underline the importance of tumor control before transplantation.





P853

Minimum tolerable interval of anti-CD20 90Yttrium ibritumomab-tiuxetan to high-dose chemotherapy with carmustin, etoposide, cytarabine, melphalan and autologous stem cell transplantation for relapsed or refractory aggressive B-NHL

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High-dose chemotherapy and autologous stem cell transplantation (ASCT) of relapsed or refractory aggressive B-cell Non-Hodgkin-Lymphomas (aNHL) are mostly ineffective in the rituximab era. Implementation of radio-immunotherapy in conditioning regimens may be a valuable option to enhance the efficacy. To achieve an optimal interaction of radio- and chemotherapy we evaluate the minimum tolerable interval of anti-CD20 90Yttrium ibritumomab-tiuxetan (Zevalin®) to high-dose chemotherapy with carmustin, etoposide, cytarabine, melphalan (BEAM) and ASCT in a prospective, multi-centric, open, non-randomized phase I/II trial.

Patients (pts) without disease progression of relapsed or refractory CD20+ aNHL during salvage therapy proceeded to Z-BEAM and ASCT. Zevalin® therapy was given with 0,4 mCi/kg body weight on day -14, day -12 or day -10 (dose levels 1-3) prior to ASCT in a 6 + 6 pts per dose level study design. BEAM was administered from day -7 to -2.

2006 to 2009 26 pts, median age 58y (34-66) received study drug. Histology included 14 diffuse large B-cell lymphomas, 6 follicular lymphomas III°, 5 transformed follicular lymphomas and one aNHL without further subtyping. In all but one case, first-line therapy contained rituximab. Median lines of prior therapies were 1 (1-4). Then, pts received 2 (2-4) cycles of salvage therapy. Median interval from diagnosis to relapse/progression was 16 months, with 11 pts progressing within one year after diagnosis. IPI at time of relapse was 0-1 in 12 pts, 2-4 in 14 pts. Overall response rate to salvage therapy was 65% and 91% after Z-BEAM. Median CD34+ cell dose was 4.2x10e6/kg (2.1-19). Engraftment showed no significant differences in the pts of dose levels 1-3. Median leukocyte recovery (>1/nl) was at day +10 (9-22). Platelets >25/nl and >50/nl were reached at days +13 (9-22) and +20 (13-53). Substitution of 3 (1-12) platelets and 4 (0-10) erythrocyte concentrates were necessary. There were two therapy-related early deaths (day +7, +18) due to infections. Zevalin® at day -10 of ASCT after BEAM was determined as highest applicable dose level. Median follow-up is 18 months (4-50). 3-y progression-free and overall survival is 59% and 73%, respectively.

90Yttrium ibritumomab-tiuxetan administered 10 days prior to ASCT after BEAM is feasible and safe. In pts who experience relapse or progression of aNHL after prior rituximab treatment, Z-BEAM and ASCT results in a high response rate with probably promising durable disease control.

P854

A novel high-dose therapy regimen with Bendamustine, Etoposide, Cytarabine and Melphalan (BeEAM) followed by autologous stem cell rescue is safe and highly effective for resistant/relapsed lymphoma patients: a phase I-II study on 44 patients

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BEAM (Carmustine, etoposide, cytarabine, and melphalan) is the most used conditioning regimen before autologous stem cell transplant (ASCT) in lymphoma patients. However, relapse rate after transplant is still a matter of concern. Therefore, new regimens with a higher efficacy are particularly needed.

We designed a phase I-II study to evaluate the safety and the efficacy of increasing doses of Bendamustine for the conditioning regimen to ASCT for resistant/relapsed lymphoma patients. As a biological background, we performed in vitro experiments which showed the synergistic activity of

bendamustine with etoposide, aracytin and melphalan in lymphoma cell lines.
Forty-four patients (median age 47 years) with resistant/relapsed non-Hodgkin (29) or Hodgkin (15) lymphoma were consecutively enrolled in the study. The new regimen consisted of increasing doses of Bendamustine coupled with fixed doses of Etoposide (200 mg/m²/day on days -5 to -2), Cytarabine (400 mg/m² on days -5 to -2) and Melphalan (140 mg/m² on day -1) (BeEAM regimen). The study was registered at EMEA with the EUDRACT no 2008-002736-15. The starting dose of Bendamustine was 160 mg/m²/daily given on days -7 and -6, which was then escalated according to the Fibonacci's increment rule until the onset of severe adverse events and/or the attainment of the expected MTD, but not higher than 200 mg/m².
The administration of Bendamustine was safe in all the 3 cohorts of 3 patients. We then fixed the dose of Bendamustine 200 mg/m² as safe and effective for the Phase II study.
A median number of $5.68x10^6$CD34+/kg cells (range 2.4-15.5) collected from peripheral blood was reinfused to patients. All patients engrafted, with a median time to ANC>$0.5x10^9$/l of 10 days. Median times to achieve a platelet count >$20x10^9$/l and >$50x10^9$/l were 13 and 16 days respectively. Twenty-two out of 44 patients presented a fever of unknown origin. All patients received G-CSF after transplant for a median time of 9 days (range: 8-25).
After a median follow-up of 14 months from transplant, 37/44 patients are in complete remission. Five out of 44 patients relapsed after a median time of 3 months from transplant, whereas 2/44 did never achieve a remission and rapidly died due to progressive disease. Remarkably, 4/39 patients achieved the first complete remission after receiving the high-dose therapy with ASCT.
In conclusion, the new BeEAM regimen is safe and highly effective for the treatment of highly resistant lymphoma patients.

P855

Autologous haematopoietic stem cell transplantation in elderly patients with lymphoma: French Society of Bone Marrow Graft Transplantation and Cellular Therapy experience

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Aim of the study: Autologous hematopoietic stem cells transplantation (AHSCT) is clearly a crucial therapeutic strategy traditionally in lymphoma patients younger than 65 years. We here propose to evaluate the toxicity related morbidity (TRM) of AHSCT in elderly lymphoma patients.
Patients and methods: Lymphoma patients older than 70 years who had undergone AHSCT performed between 1995 and 2009 were retrospectively analyzed.
Results: A total of 80 AHSCT were included. Datas had been completed for 62 patients, 43 men (69%) and 19 women (31%). Diagnosis were diffuse large B-cell (n=20, 33%), follicular (n=15, 25%), mantle cell (n=11, 18,3%), T-cell (peripheral n=3, 5%; angioimmunoblastic n=1, 2%; anaplastic large cell n=1, 2%), aggressive B (Burkitt n=2, 3%; centroblastic n=2, 3%) and other type of high grade (n=7, 11%) lymphomas. Median delay between diagnosis and AHSCT was of 24.3 months [4.8-435.4] and median age at transplant was of 72.3 years [70-80]. At transplant, 53 patients (85%) were in complete response, one patient in stable disease (2%) and 7 were in relapse (13%). Thirty three (53%) presented comorbidities. A median of 1 [0-4] treatment line was previously received. Major graft source was PBSC (n=61, 98%) (Median CD34 dose infused of $3.7.10^6$/kg [1.4-16.1]). Main conditioning were BEAM (n=42, 68%) and melphalan (n=10, 17%).
Median delays to reach 0.5.109/L neutrophils, 20 and 50.103/L platelets were of 12.1 days [7.9-22], 12 [6.1-55] and 16 [9.1-826.5] days respectively. Five patients had never reached these platelets threshold. Twenty four (39%) and 14 (23%) patients presented infection and non infection related complications respectively. Median hospitalization duration was of 14.5 days [10.9-28.9] (6 patients (10%) were in a convalescence house).
At 3 months post transplant, survival rate was of 92%. Two patients died of TRM (3.2%) and 3 of relapse (4.8%). Sixty four percents of patients presented a good overall health and 54% a stable or improved body mass index.
With a median follow-up of 69.8 months [12.4-183], median overall survival was of 55 months [0-84]. BEAM conditioning was correlated with better survival (55 months versus 12 months, p=0,047). Number of previous treatment lines, comorbidities, CD34 infused and status at transplant had no prognostic value in our cohort.
Conclusions: AHSCT seems to be a feasible procedure in elderly patients with lymphoma. However, TRM remains high.

P856

Unmanipulated haplo-identical bone marrow transplantation (following non-myeloablative conditioning) for advanced Hodgkin's lymphoma using post-transplantation cyclophosphamide

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High dose, post- transplantation Cy promotes tolerance in alloreactive host and donor T cells and is effective as prophylaxis of GVHD. The safety and efficacy of posttransplantation Cy after nonmyeloablative conditioning was demonstrated by more series of recipients from haploidentical related donor with poor hematological disease.
We report our experience on 12 patients with advanced HD who underwent related haploidentical T replete bone marrow transplantation from two institutions. At transplantation, median age was 35 years (range 23 –61). All patients received non myeloablative conditioning with Cy/Fludarabine/TBI 200 rads. GVHD prophylaxis consisted of Cy (50 mg/kg intravenously) on days + 3 and + 5 and MMF + Cyclosporine (n.7) or tacrolimus (n.5).
Most patients (8/12) had refractory or active disease at BMT and all had failed autologous BMT. Sustained engraftment of donor cells occurred in all 12 patients with bone marrow chimerism 100% donor. The median time to neutrophils recovery (>500/mcl) and platelets recovery (>20.000/mcl) was respectively +19 and +25 days from BMT. No graft failure was observed. High dose of posttrasplantation Cy was well tolerated without toxicities. The incidence of grade II–IV GVHD was 8% (1 patient with cutaneous GVHD). The chronic GVHD was absent.
The absolute lymphocyte count on day +30 and +60 was respectively 100/mcl and 600/mcl. The opportunistic infections complications are: 1 pulmonary Aspergillosis,2 sepsis (E. Coli), 2 pneumonia, 1 EBV reactivation a 5 CMV infections (1 disease). With a median follow up of 9 months (range 2 – 20) all patients are alive. No patients died for TRM. Relapse incidence was 33% (4 pts), after a median time of 10 months fom BMT.
In conclusion our experience confirms that high dose post transplant CY is effective as prophylaxis of GVHD after haploidentical BMT, can prevent rejection and does not appear to eliminate the allogeneic graft versus lymphoma effect.

P857

Conditioning with treosulfan and fludarabine for patients with refractory or relapsed non-Hodgkin's lymphoma

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Background: Treatment of patients with refractory or relapsed non-Hodgkin lymphoma (NHL) remains challenging. Autologous and particularly allogeneic stem cell transplantation (allo-SCT) constitutes a curative approach but is also characterized by transplant associated mortality (TAM). Conditioning with treosulfan and fludarabine has been used for several hematological malignancies including NHL. Here we present a retrospective analysis of patients treated at the University of Rostock, the University of Jena and the University of Essen. The patients received treosulfan and fludarabine as conditioning for allo-SCT for NHL.

Material and methods: 88 patients with refractory or relapsed NHL had received a mean of 2.6 (range: 1-7) different chemotherapy regimens, 47/88 a preceding autograft. 22 patients suffered from diffuse large B-cell NHL, 22 from CLL, eight from mantle cell lymphoma and seven from follicular lymphoma. 29/88 patients were at relapse with 17/88 early relapses (<6 months). Eleven patients with refractory disease were observed. 25 patients were in complete (CR), 42 patients in partial remission (PR). The rest of the patients had stable or progressive disease. 39 patients solely received allo-SCT, 47 patients an autologous followed by an allogeneic graft and two patients received two allogenic grafts.

Results: 69 of 88 patients achieved a CR, seven patients a PR. Three patients with progressive disease and two with stable disease were observed. 45 of 88 patients died, 21 due to infectious complications, four due to graft-versus-host disease (GVHD). Three patients died because of progressive disease and three patients due to infections and progressive disease. Seven patients relapsed and seven patients died because of reasons not related with the lymphoma. In total 54 patients developed acute GVHD and 40 patients chronic GVHD. For all 88 patients after 3 years the probability of overall survival (OS) was 50% and the disease free survival (DFS) was 44%.

Conclusion: Allogeneic stem cell transplantation following conditioning with treosulfan and fludarabine constitutes a therapeutical option for patients with refractory or relapse NHL after chemotherapy. To minimize the TAM and to optimize the response to treatment allo-SCT should be considered early. Patients in CR after rescue chemotherapy might profit from bypassing auto-HSCT and from directly receiving an allograft. This approach needs to be evaluated in further prospective clinical trials.

P858

Immunotherapy with FBTA05 (Bi20), a trifunctional anti-CD3 x anti-CD20 antibody in recurrent childhood B-cell malignancies

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Since treatment options for B cell malignancies in children refractory to standard treatment are limited, novel treatment approaches are required. Here we report first clinical data on FBTA05, a trifunctional anti-CD3 x anti-CD20 antibody in recurrent childhood B-cell malignancies.

Three children with relapsed diffuse large B cell lymphoma (DLBCL), one child with mature Burkitt lymphoma (BL) and three children with B-cell acute lymphoblastic leukemia (B-ALL)-relapse were treated with escalating doses of FBTA05. All children were extensively pretreated and presented refractory to standard treatment (radiation, chemotherapy) including rituximab. Five children received HLA-identical allogeneic stem cell transplantation (allo-SCT) before FBTA05 treatment.

For safety reasons, dose escalation of FBTA05 started with 10 microgram followed by 20 and 50 microgram every third day (safety part). Thereafter weekly applications of escalating doses of FBTA05 (100-300 microgram) were performed. In two patients the safety part was followed by donor lymphocyte infusions (DLI) and repeated once or twice in four week intervals. Due to tumor progression in 3 patients, FBTA05 was escalated daily up to 200, 500 and 1000 microgram, respectively.

In terms of safety and toxicity, 6- to 12-hour infusions of dose-escalated FBTA05 were well tolerated by all children. Adverse events were restricted to fever and chill and could be managed by supportive treatment. Repeated applications with escalating doses did not result in increased severity of adverse events. In only one patient, human anti-mouse antibodies (HAMAs) were detectable 4 weeks after start of treatment. Importantly, this patient could be safely treated with two additional applications of FBTA05. The cytokine profile was characterized by transient increase of IL-6, IL-8 and IL-10. With the exception of one patient dying due to fulminant disease progression, all children showed clinical response: five stable diseases and one complete remission. The survival up to now ranges from 48 to 483 days (to be updated at time of presentation). Graft versus host disease (grade III-IV) developed in two patients (in one case after DLI), but could be controlled by further immunosuppressive therapies.

Taking into account the progressive disease and the high risk for progress and death after allo-SCT, FBTA05 resulted in an astonishing response in 6 out of 7 children either alone or in the context of allo-SCT with or without subsequent DLI.

P859

High-dose sequential chemoimmunotherapy supported by autologous stem cell transplantation in patients with systemic B-cell lymphoma and central nervous system involvement: interim analysis of a multicentre phase II Trial

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Background: CNS dissemination is a fatal event in aggressive lymphomas. Encouraging results with high-dose chemother-

apy + ASCT have been reported in a few, small retrospective studies. We designed a multicentre phase II trial to evaluate feasibility and activity of an intensified chemoimmunotherapy combination supported by ASCT in pts with B-cell lymphoma and CNS involvement (SCNSL1 trial). Herein, we report the interim analysis.
Methods: HIV-negative pts (≤70 ys; ECOG PS ≤3) with B-cell lymphoma and CNS dissemination at diagnosis or relapse were enrolled. Experimental treatment was: Induction: methotrexate 3.5 g/m^2 d1, cytarabine(araC) 2 g/m^{2}x2/d d2-3; Consolidation (R-HDS): sequential administration (every 3 weeks) of cyclophosphamide (CTX) 7 g/m^2; araC 2 g/m^{2}x2/d d1-4; etoposide 2 g/m^2; Conditioning: BCNU 400 mg/m^2 d-6, thiotepa 5 mg/kg d–5-4 and ASCT d0. Treatment included 5 doses of rituximab and 4 doses of intrathecal liposomal cytarabine. Pts with residual disease after ASCT received whole-brain irradiation. Two-year PFS is the primary endpoint (ClinicalTrials.gov NCT00801216).
Results: The first 17 registered pts (median age 52 ys, range 36-70; M/F ratio: 1.1) were considered. Nine pts were treated at diagnosis and 8 at relapse; lymphoma histotypes were diffuse large B-cell (n=14), mantle cell (2) or follicular. Ten pts had concomitant systemic disease; no pt had positive CSF.
All pts completed induction, 12 pts completed R-HDS (ongoing in other 3), 8 pts received ASCT. G4 hematological toxicity was reported in all pts; no cases of G4 non-hematological toxicity were recorded; one pt died of septic complications. Peripheral blood SC collection after CTX or araC was successful in 12 of the 15 referred pts (median 10 x 10^6/kg; range 7.2-18.9). One poor-mobilizer pt received sibling SCT.
After induction, CNS response was complete in 6 (35%) pts and partial in 10 (ORR=94%). At the end of the program, CNS response was complete in 11 (65%) pts and partial in 2 (ORR=76%). Eight of 10 pts achieved a remission of systemic disease. At a median follow-up of 12 months, 6 pts experienced failure, with a one-yr PFS of 60±14%; causes of death were lymphoma (n=6; CNS in 5) or sepsis (2), with a one-yr OS of 66±13%.
Conclusion: This intensified therapeutic program is feasible in pts ≤70 ys with systemic B-cell lymphoma and CNS dissemination. G4 toxicity is exclusively hematological and manageable. Interim results warrant proceeding with planned accrual (n=38).

P860
TEAM (Thiotepa, Etoposide, Cytarabine, Melphalan) as conditioning regimen for lymphoma treatment with autologous haematopoietic stem cell transplantation
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Background: Standard conditioning regimen before an AHSCT for chemosensitive relapsed Hodgkin Lymphoma (HL) and Non Hodgkin Limphoma (NHL) patients (pts) is still represented by a scheduled combination of carmustine, etoposide, cytarabine and Melphalan (BEAM). Despite the efficacy of this regimen, it is disadvantaged by the presence of carmustine, which is responsible of a high pulmonar toxicity with fibrosis and progressive reduced diffusion capacity. In order to prevent a significant side effect, we replaced carmustine with Thiotepa in 81 relapsed and refractory HL and NHL pts. We have also evaluated the efficacy and tolerability of this new regimen.
Patients: At day -7 81 pts received 10 mg/kg thiotepa (5 mg twice every 12 hours) followed by cytarabine 200 mg/m^2 die (-5 to -3) etoposide 200 mg/m2 die (-5 to -3) and melphalan 140 mg/ m2 (day -2). Out of these pts, 51 were male and 30 female. Forty-eight pts were affected by da NHL and 33 by HL. At the moment of transplant 30 (37%) pts were in CR, 43 (53%) were in PR and 8 (10%) were in progression. All patients were in second or sebsequent line of therapy. The median time of neutrophil >500 and platelets >20000 recovery was of 10 (range 8-12) and 12 (range 10-20) days respectively. One patients died of severe infectious event. Twenty-seven pts maintain the CR, 30 have reached CR, 6 have a stable disease and 18 pts did not respond to the treatment. At last follow up 70 (85%) patients are alive and 50 of them in CCR with a median follow-up of 18 months (3-41). Ten patients succumbed to their disease Conclusions: Our experience with TEAM protocol has shown a significant efficacy not lower to our historical experience with BEAM protocol. The tollerability was significantly accetable and only one serious adverse event has been documented during the treatment or during aplastic phase, and the TRM at 100 days is 1,2%. In no case, oral mucositis was higher than grade 2, even if most of the pts underwent total parenteral nutrition. Finally no cases of serious organ toxicity was observed (pulmonar, hepatic, cardiac and renal). Hematopoietic recovery, stimulated with G-CSF, has been reached as scheduled and no cases of prolonged neutropenia were observed.

P861
Mantle cell lymphoma and autologous stem cell transplantation: no difference in term of outcome regarding the use or not of TBI in the conditioning
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Mantle cell lymphoma (MCL) is a B-cell non-Hodgkin's lymphoma (NHL) characterized by overexpression of cyclin D1. It's among the most aggressive NHLs entity with a median overall survival (OS) of 6 years. Intensive chemotherapy followed by autologous stem cell transplantation (ASCT) upfront provides high complete remission rate. However, the question of the best conditioning regimen in ASCT is still pending. One major issue is the role of TBI (Total Body Iradiation). To answer this question, the outcome of all the MCL patients (pts) (n=73) who underwent ASCT in our institution from 1990 to December 2008 were retrospectively analyzed in regard of the use or not of TBI as part of the conditioning. There were 50 males and median age at diagnosis was 54 years (34-69). All pts at diagnosis presented with stage III-IV. MCL international prognostic index (MIPI) was ≤ to intermediate risk in 51 cases and ≥ to intermediate risk in 22 cases. Nineteen percent of the pts had a blastoid feature. Sixty-seven pts received ASCT upfront; 4 underwent ASCT at first relapse and 2 at second relapse. Prior ASCT, pts received (R)-CHOP(-like) (n=36), (R)-DHAP (n=22) or (R)-DHAP+(R)-CHOP (n=12). Disease status at the time of transplantation was partial response (PR) in 52 cases and complete remission (CR) in 20 cases. A conditioning regimen including TBI was given to 44 pts (TBI group), while 28 pts received BEAM (non-TBI group). According to sex, age, Ann Arbor stage, extra-nodal involvement, MIPI, blastoid feature and the number of lines of chemotherapy prior ASCT, there was no statistical difference between the two groups. After ASCT, 63 pts reached CR (37 in the TBI group compared to 26 in the non-TBI group) and 5 PR (3 in the TBI group compared to 2 in the non-TBI group). The median follow up (FU) from time of diagnosis is 3,2 years (range: 0.75-13.3) and 2.5 years from time of ASCT (range:

0.22-12.9). After ASCT, the 1- and 3-year OS rates are 90 and 70% and the event free survival (EFS) rates are 84 and 58%, respectively. In bivariate analysis, the 1- and 3-year OS rates are similar in the non-TBI vs the TBI groups: 89% vs 90% and 71% vs 70% (p=0.82), respectively. The comparison between the 2 groups shows also no difference in term of 1- and 3-year EFS rates (85% vs 83% and 55% vs 58%; p= 0.89). CONCLUSION: The present analysis shows no advantage in the use of TBI for the conditioning regimen prior ASCT in the setting of MCL pts.

P862

Outcome of Hodgkin's lymphoma and aggressive non-Hodgkin lymphoma patients refractory to salvage chemotherapy

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Background: The outcome of lymphoma patients refractory to second line salvage chemotherapy (CT) is dismal and most of these patients finally died from disease progression. The aims of this retrospective study was to define the overall response rate (ORR), overall survival (OS), and progression free survival (PFS) obtained after two different third line salvage chemotherapy (conventional CT vs HDC) in a cohort of patients with disease refractory to second line salvage CT.

Patients and methods: From March 2000 to January 2009, 76 lymphoma patients relapsed and refractory to first line CT, were treated with salvage CT. Of these patients, 42 patients (55%) were stable or progressive after the second line salvage CT. Second line CT was VIHA [(vinorelbine (25 mg/m², day 3), cytarabine (2 g/m²/day, day 1 and 2), and ifosfamide (2.5 g/m²/day, days 1-3, associated or not to Rituximab (375 mg/m²/day)] in NHL patients (n= 24) and IGEV [(vinorelbine (20 mg/m²/d, day 1 and 4), gemcitabine (800 mg/m²/d, day 1 and 4), and ifosfamide (2 g/m²/d, day 1-4)] in HL patients (n=18). The third line salvage CT consisted of conventional CT (n=24), or high-dose chemotherapy (HDC) (n=18).

Results: The median observation time was 57.0 months (range 2-112), and the ORR was 66% after HDC compared to 8% after conventional-CT (p 0.001).

The overall 3-year and 5-year OS, was 30% and 25%, and PFS was 19% and 17%, respectively. The median OS and PFS were 13 and 10 months, respectively. However, when conventional CT was compared to HDC, the 3-year OS and PFS (20% vs 44% and 8% vs 33%, respectively), and 5-year OS and PFS (15% vs 39% and 0 vs 28%, respectively) were significantly in favour of the HDC-group (p= 0.01 and 0.051). Most of patients died from lymphoma progression (85%). In addition, the 15 patients who received alloSCT had a significantly better 3-year OS and PFS [53% vs 17%, (p=0.01) and 47% vs 4% (p=0.008), respectively].

Conclusion: In this retrospective study the outcome of NHL and HL, in terms of ORR and survival, was significantly better when the third line salvage therapy consisted of HDC which in turn allowed more patients to proceed to alloSCT. These results seems to indicate the HDC is an effective therapeutic option even in advanced lymphoma patients, however, these data need to be prospectively confirmed.

P863

Allogeneic stem cell transplantation with an unrelated donor for Hodgkin's lymphoma: a systematic review

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Objectives: Allogeneic stem cell transplantation (allo-SCT) is regarded as a clinical option or a developmental approach for patients with Hodgkin's lymphoma (HL) with primary refractory or relapsed disease. The aim of the current project was to systematically review the available evidence and determine the benefits and risks of allo-SCT with an unrelated donor (UD) for adult patients with HL.

Methods: We performed a systematic search in bibliographic databases (EMBASE, MEDLINE, PubMed, and The Cochrane Central Register of Controlled Trials [Clinical Trials]). Allo-SCT with an UD was to be compared with conventional chemotherapy or autologous SCT, and different allo-SCT strategies were also to be evaluated. The following patient-relevant outcomes were to be analysed: overall (OS) or progression-free survival (PFS), therapy-related complications, health-related quality of life, and psychosocial aspects. Only trials published in full text were considered for evaluation.

Results: 8 studies with 454 patients could be included in the analysis. None of these studies was an RCT, 3 were non-comparative and 5 provided results for a comparison of allo-SCT with an UD versus a related donor. Data on OS showed no consistent tendency in favour of a donor type. The same applied to PFS. Nor did the results on therapy-associated mortality, acute (grade II-IV) and chronic GvHD provide a uniform picture. Because of the heterogeneity between studies meta-analyses were not performed. No useable data could be extracted with regard to further serious therapy-related complications, secondary neoplasm, or health-related quality of life and psychosocial aspects. No studies were identified for the other research questions, e.g. comparison of allo-SCT with an UD versus conventional chemotherapy or autologous SCT.

Conclusions: Current evidence is sparse and does not allow a reliable appraisal of the benefits and risks of allo-SCT with an UD for patients with HL. However, studies including UD but not analysed according to donor types show that allo-SCT with reduced-intensity conditioning substantially improves OS compared to chemo- and/or radiotherapy alone. Therefore, it is particularly evident that separate analyses according to treatment arms (related and unrelated donors) are required in such studies. If allo-SCT with an UD is performed in individual cases, patients must be informed about the uncertain evidence base.

P864

Treosulfan/fludarabine/thiotepa conditioning for patients with advanced malignant haematologic lymphoproliferative diseases

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Reduced-intensity and reduced toxicity conditioning regimens for allogeneic stem cell transplant (HSCT) are accepted for the treatment of high risk patients non fitting criteria for an ablative approach. New and old drugs have been tested to find a less toxic but efficient preparation. The association Treosulfan- Fludarabine has demonstrated to be well tolerated and myeloablative; we added Thiotepa to this combination with the aim to increase the anti-tumor activity in a subset of malignant advanced lymphoproliferative disease. Primary objective was to evaluate feasibility and safety, secondary objective was evaluation of OS,DFS, TRM and relapse.

Since November 2006 to September 2010, 13 patients (8 males, 5 females) entered this study.

Median age was 41 years (range 19-60). Underlying diseases were: NHL (7), HD (4), Acute Biphenotypic leukemia (1), CLL (1). All patients were pretreated in advanced phase of disease; no patient was in 1st complete remission; 5 were in 2nd CR, 1 in 3rd CR, 1 in PR, and 6 in resistant/progressive disease. Mean HCT-CI was 1.
Conditioning consisted of Treosulfan 14 gr/mq for 3 days, Fludarabine 30 mg/mq for 5 days and Thiotepa 10 mg/Kg single day. Cyclosporine/short MTX were used as GVHD prophylaxis and anti-Lymphocyte globulins (Thymoglobulin or ATG Fresenius) were used in case of MUD transplants.
Nine (9) patients received HSCs from HLA identical siblings and 4 from match unrelated donors. Source of stem cells were bone marrow in 5 patients and peripheral blood stem cells in 8.
Twelve (12) patients (92%) regularly engrafted. One (1) patient did not engraft (an 18 yo girl with advanced refractory pulmonary HD who died by respiratory failure).
Four (4) patients experienced GI toxicity (3 grade III mucositis, 1 GI bleeding), one (1) patient had grade I renal toxicity, one (1) patient presented CNS haemorrhage. Six (6) patients presented grade 1 acute GVHD. CrGVHD occurred in 5 patients (limited in 4, extensive in 1).
Seven (7) patients are alive (53.8%) in complete remission with a median follow-up of 16 months (range 2-39 months). Six (6) patients died (46%): 2 for recurrent disease, 4 for TRM.
These preliminary data underline that Treosulfan-Fludarabine-Thiotepa can be safely used for allogeneic conditioning regimen in patients with advanced lymphoproliferative disease. Engraftment is regular; post transplant toxicity is reduced compared to the standard conditioning. The role of Thiotepa seems important in increasing the anti-tumor effect of this regimen.

P865
High-dose chemotherapy and autologous stem cell transplantation for primary refractory Hodgkin's lymphoma: outcome and prognostic factors in 86 patients of a single institution
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Although high-dose chemotherapy (HDC) with autologous stem cell transplantation (ASCT) is the standard treatment for chemosensitive primary refractory Hodgkin's lymphoma (PRHL), there is no established therapy for chemoresistant PRHL. We retrospectively analyzed efficacy and prognostic factors in 86 patients [48M/38F, delta m age 25 years (14-56)] with chemosensitive/chemoresistant PRHL who underwent HDC+ASCT from 1994 to 2009. Baseline characteristics included: stage III/IV=52 (60%), nodular sclerosis histology=61 (71%), b-symptoms=55 (64%) and bulky disease=26 (30%). All patients showed resistance or progression after first-line treatment (ABVD) and received DICE (Dexamethasone+ICE) as salvage. Patients achieving complete remission (CR) or ≥50% response (PR) after DICE were defined as chemosensitive. Chemosensitive patients (24/86) proceeded to HDC-ASCT, while from the chemoresistant (62/86), 19 were administered HDC-ASCT and 43 received other regimens for de-bulking before ASCT (24=1 regimen kappa α Iota 19> 2 regimens). delta m period from diagnosis to ASCT was 13 (7-25) months and the status before ASCT was: CR (n=12), PR≥50% (n=45), resistance/progression (n=29). BEAM was the main conditioning regimen. The estimated 10-year overall survival (OS) and disease-free survival (DFS) was 61% and 55% (delta m follow-up=5.2 years). The estimated 10-year OS for chemosensitive and chemoresistant patients was 75% and 52%, respectively (p=ns) while the estimated 10-year DFS was 65% and 51%, respectively (p=ns). Multivariate analysis revealed as negative prognostic factors for the outcome of ASCT, the elevated erythrocyte sedimentation rate (ESR>20), the age<20 years and the administration of ≥2 treatment-lines after DICE. Our data suggest that resistance to salvage treatment does not negatively affect the outcome of ASCT in patients with PRHL, when less than 2 (0-1) additional salvage regimens are administered before ASCT. The elevated ESR and young age are negative prognostic factors for the outcome of ASCT in PRHL. HDC+ASCT could be considered for and would likely benefit a non-negligible number of patients with PRHL.

P866
Primary mediastinal B-cell lymphoma: results of autologous stem cell transplantation as consolidation treatment in 20 patients with long follow-up. A single-centre experience
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Introduction: Primary mediastinal B-cell lymphoma (PMBL) is an entity which clinical features, molecular analysis and gene expression profile have identified it as a distinct subgroup of nodal diffuse large B-cell lymphomas. Usually it is confined to the mediastinum where it presents very aggressive local behaviour. Therefore, biological data and the International Pronostic Index (IPI) are very limited to identify patients (p) in great risk of relapse. Initial therapy is critical in treating PMBL because salvage therapy for recurrence is of limited efficacy. The optimal therapy remains undefined and the role that the autologous stem cell transplantation (ASCT) plays still is on debate in the "rituximab era". We present our experience with ASCT in the treatment of PMBL.
Methods: We analyze retrospectively 20 p treated consecutively in our center for a period of 14 years (y), (1996-2010). 13 were women and 7 were men with a mean age of 33,4 y. Of these patients, 17 presented Ann-Arbor staging I-II and 3 staging III-IV. 8 of the p presented systemic ("B") symptoms and 2 were IPI>2. 14 p were treated with CHOP like regimens, 6 with third generation anthracycline containing regimens and all of them with mediastinal involved-field radiation therapy and ASCT with BEAC o BEAM conditioning regimen. Since 2003, rituximab has been used with the initial regimen (7 p).
Results: After a median follow-up of 86 months (range, 9–163 m), 18 of 20 p (90%) were alive without disease. 14 were in complete remission (CR), 4 in partial response (PR) and 2 in refractory situation when ASCT was performed. All chemosensitive p (18) reached and/or mantained CR after ASCT till now. The 2 p that were refractory, died of disease progression 1 and 3 months after ASCT. The estimated disease-free survival and overall survival mean was 11,8 years. No secondary neoplasms were diagnosed during the follow-up.
Conclusion: Our report confirms the efficacy of ASCT in patients with PMBL. Failing to define a prognostic scale applied to PMBL, the role of FDG-PET in the evaluation of response to treatment and the impact of rituximab in the initial scheme of chemotherapy, ASCT as consolidation treatment should be advised to some of the patients. Randomized trials are needed to clarify these issues.

P867
Reduced-intensity allogeneic stem cell transplantation for relapsed/refractory Hodgkin's lymphoma: no benefit of graft-versus- lymphoma effect
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Background: There are few treatment options for patients with multiple relapsed or refractory Hodgkin lymphoma. Allogeneic

transplantation has been associated with high TRM and there is little evidence for clinical graft-versus-lymphoma effect.
Objective: To evaluate the safety and efficacy of reduced-intensity conditioning for allogeneic stem cell transplantation in patients with relapsed or refractory Hodgkin lymphoma.
Methods: Prospective study conducted in the bone marrow transplant center (CEMO) of the Instituto Nacional de Cancer (INCA) in Rio de Janeiro, Brazil, from December 2000 to February 2009. Conditioning regimen consisted of fludarabine (25 mg/m² for five days) and cyclophosphamide (60 mg/kg for two days). Rabbit ATG (2 mg/kg for four days) was added for unrelated transplantation. GVHD prophylaxis consisted of cyclosporine and methotrexate (10 mg/m² on D+1 and 5 mg/m2 on D+3 and D+6).
Results: Twenty-one patients were included, eighteen with sibling donor and three with unrelated donor. All but one had failed a previous autologous transplant. Fourteen patients were chemorefractory, while six had sensitive disease. One had an untreated relapse. All related transplants were done with peripheral blood stem cell graft. Bone marrow was the graft source in one unrelated transplantation, and cord blood in two. Eleven patients achieved complete quimerism, four achieved mixed quimerism, one had no quimerism and it was not assessable in five. Acute GVHD grade II to IV occurred in seven patients (33%). Two developed limited cGVHD (9%) and seven extensive cGVHD (33%). DLI was given to five patients. Duration of response was partial and brief. All patients died, six of them before D+100 (28%). In thirteen patients (62%), relapse was the cause of death. The mean and median time of survival was 531 and 348 days respectively (range 16 to 1737 days).
Conclusion: This study shows that there is no improvement in survival of patients with advanced Hodgkin lymphoma by allogeneic transplantation with reduced-intensity conditioning despite extensive cGVHD in some patients. This result, in contrast to others, is probably because of the large proportion of patients who were chemorefractory. GVL effect does not benefit this group of patients. New forms of therapy should be evaluated.

P868
Autologous haematopoietic stem cell transplantation is highly effective for patients with diffuse large B-cell lymphoma failing upfront R-CHOP regimen
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Background: Diffuse large B-cell lymphoma (DLBCL) remains one of the most frequently seen non-Hodgkin lymphoma (NHL) with an aggressive disease course. Only 40-50% of patients (pts) may be cured with chemo - and radiotherapy; the remaining patient subset remains partially chemosensitive or resistant.
Material and methods: Between January, 2000 and December, 2010, we conducted AHSCT in 25 pts with DLBCL who failed upfront R-CHOP regimen. There were 15 male and 10 female, median age of 52 (range 23-66 yrs). Ann Arbor staging at diagnosis was as follows: II-(n=4), III-(n=3), IV-(n=18); 80% of pts manifested B-symptoms. 61% of pts had an aged-adjusted IPI 2 or 3. Initially, all pts received 6 cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) and achieved partial response which was defined as the reduction of measurable disease by ≥50% without the appearance of any new lesions. Pts with PR proceeded to high dose chemotherapy (HDT) followed by AHSCT.
Results: In 23 pts stem cells were collected from peripheral blood after IVE chemotherapy (ifosfamide, etoposide, epirubicine) and subsequent administration of G-CSF, 10 ug/kg/d, starting from +5 day after chemotherapy. G-CSF alone was used in 2 remaining pts. Collections were performed using Optia Spectra. All pts collected the sufficient number of CD34+ cells for AHSCT. Conditioning regimens before AHSCT consisted of CBV (BCNU, Etoposide, Cyclophosphamide) in 21 cases, BEAM (BCNU, Etoposide, Ara-C, Melphalan) in 3 and LACE (CCNU, Etoposide, Ara-C, Cyclophosphamide) in one. A median number of transplanted CD34+ cells was 9.5 (1.33-35.76x10^6/kg). All pts successfully engrafted. Hematopoietic recovery was as following: WBC>1,0x10^9/L after median of 12days (range 9-15), ANC> 0,5x10^9/L after median of 13days (range 9-16) and PLT>20x10^9/L after median of 13days (range 7-20). All except two pts achieved complete remission after AHSCT. These two pts underwent second AHSCT and achieved complete remission. The major complications after AHSCT were rare and included: bacterial infections (n=3), viral conjunctivitis (n=1), oral mucositis (n=2). At the last contact, 24 pts are alive in CR with a median follow-up period of 62 months (range 6-120). One patient died two years after AHSCT due to disease progression.
Conclusions: HDT followed by AHSCT seems to be highly effective and safe procedure in DLBCL patients, who failed the upfront R-CHOP regimen.

P869
Possibility of favourable outcomes among refractory to salvage-therapy Hodgkin's lymphoma patients
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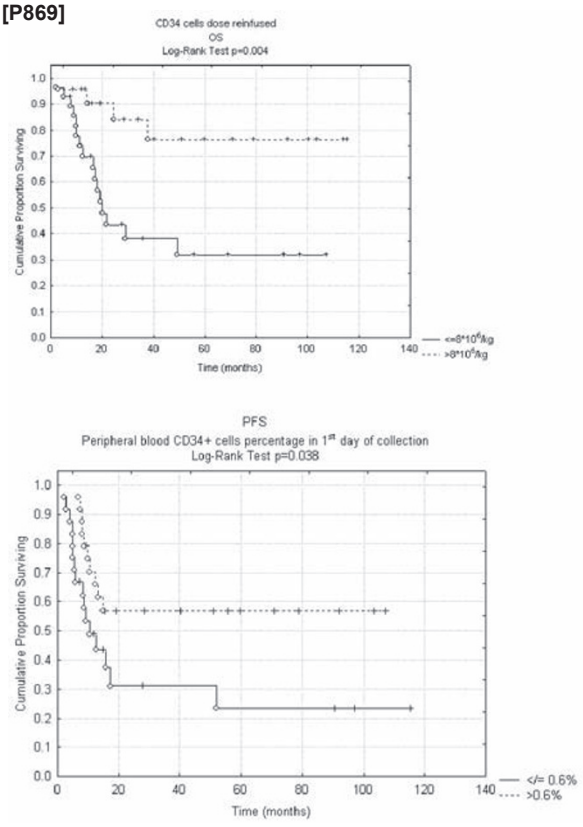
Purpose: Response to salvage therapy (tumoral chemosensitivity) is a cornerstone factor which influences to enrolment in the HDT and ASCT program for relapsed/refractory Hodgkin's lymphoma. We report here retrospective analysis evaluating the outcomes of 51 pts who didn't response to salvage therapy or progressed before ASCT. HDT is controversial for chemorefractory group but it hasn't contradicted our transplant program.
Patient and methods: In total, 26(51%) males and 25 (49%) females with chemorefractory HD after salvage were treated with HDT and auto-SCT from Feb 2001 to Oct 2010. The median patient age was 29.2 years (range 13-54). There were 26 primary-refractory pts (51%) before salvage, others relapsed. The average number of cycles of previous therapy was 11.9 (range 6-32); 38 pts (74.5%) underwent radiotherapy; 69.4% pts had B- symptoms before salvage. Extranodal involvement was observed among 32 pts (62.7%). Salvage regimens were mainly platinum-based; they were received by 48 pts (94%). 14 pts (27.4%) received more than one line pretransplant salvage therapy.
Among included not responding patients, 14 (27.4%) were progressing; others had a stable disease before HDT. 56 auto-SCT and one allo-SCT were performed. Median numbers of reinfused CD 34+ cells were 6.94*10^6/kg (range 1.97-18.8*10^6/kg). Most frequently used conditioning regimens were CEAM (lomustin 400 mg/m² instead carmustin) and BEAM – 48 pts (84.2%).
Results: After a median follow-up of 19.9 months (range 2.2 – 115.5) the overall survival (OS) is 69.2% (95% CI, 56%- 83%), and progression free survival (PFS) is 42.6% (95% CI 30%-56%). TRM for this category of difficult patients was 5.9%.
In the univariate analysis, the following factors were significant predictors to improve both OS and PFS: B-symptoms lack before salvage, CD 34 cells dose more than 8.0 *10^6/kg reinfused, and peripheral blood CD 34 cells percentage on 1st day of collection. Significantly better (p=.008) survival (OS and PFS) was observed among patients whose peripheral blood CD 34 cells percentage on 1st day of collection was more than 0.6%.
Conclusions: This prognostic unfavorable category of patients can also be included in transplant programs. The ability to progenitor's mobilization can influence an outcome.

[P869]



P870

Prognostic value of pre-transplantation positron emission tomography in lymphoma patients

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Objectives: We investigated the value of fluorine 18-fluorodeoxyglucose positron emission tomography ([18F]FDG-PET) to predict clinical outcome after high-dose chemotherapy combined with autologous hematopoietic stem cell transplantation (HDT/SCT) in patients with Hodgkin's and non-Hodgkin's aggressive lymphoma.
Methods: Pretransplant PET and CT were performed in 57 patients (37 patients with Hodgkin's lymphoma, 20 patients with aggressive non-Hodgkin's lymphoma). 36 patients were PET negative before HSCT and 21 patients were PET positive despite salvage second line chemotherapy (DHAP, ICE or gemcitabine-containing regimen). According CT data 19 patients were in CR, 23 patients – in PR, 6 patients had stable disease and 9 patients had progressive disease before HSCT.
Results: The median follow-up period was 1 year 10 months. Among 23 patients with PR-7 (30%) demonstrated metabolic activity in lymph nodes and 16 (70%) were PET negative, to compare with 83% and 17% in patients with stabilization before HSCT. All patients with CR according CT were PET negative. All patients with progressive disease were PET positive. The 3-year overall survival (OS) was 100% for patients with complete response according CT data, 67% for patients with partial response before HSCT, and 39% for patients with stable or progressive disease at the moment of transplantation (p= 0,021). PET negative patients had OS - 87%, whereas PET –negative patients demonstrated 43% OS (p=0.013).
Conclusion: FDG-PET is powerful prognostic factor for patients with PR or stabilization undergoing autoHSCT.

P871

Comprehensive geriatric assessment of elderly patients with lymphomas candidate to autologous bone marrow transplantation. A single-centre experience

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Introduction: ABMT is considered the treatment of choice in relapsed/refractory (R/R) Lymphomas (L). However patients (PTS)≥60 years are often not considered for this approach because of advanced age or/and the presence of co-morbidity. In addition Karnofsky index, used in oncology, are not sensitive for co-morbidity and do not correlate well with Comprehensive Geriatric Assessment(CGA composed of: ADL, IADL, CIRS-G, MMS, GDS, Geriatric Syndrome). In our Institution with the use CGA we evaluated 23 consecutive Elderly pts (EP≥60 years)with lymphoma after first line or salvage treatment to establish reproducible criteria for selecting pts for transplant.
Materials and methods: Median age was 63 years (60-75), male were 10 (43%) and female 13 (57%). Histology was as follows: DLBCL 9 (39%), Follicular G1 and G2 5, Mantle Cell 4, Composite 2, Lymphoblastic 1, PTCL 1 and Hodgkin 1. 14 were relapsed (61%), 4 refractory and 4 in consolidation. All pts were treated previously with doxorubicin or analogs and rituxan if the lymphoma was CD20+ (19 pts/82%).
Results: As salvage treatment we used R-DHAOX or R-ESHAP (15 pts/65%) prevalently. Median CD34+ collection was 4,6 x10^6/kg (1,5-17). CGA was calculated to assess the fitness status of pts: 21(91%) were fit and 2 unfit. No pt was considered frail. We assessed also the Sorror HTC-CI: 20 pts (87%) were at low risk and 3 at intermediate risk. Before ABMT 15 pts were in CR and 8 in PR. Conditioning regimen were R-BEAM or BEAM for all pts (Reduction dose to 75% for Pts>70 years). Median CD34+ reinfused was 3,81x10^6/kg (0,81-12,4). Neutrophylic recovery was in +10 (7-18) and in +12 (10-36) for platelets. Major toxicity were Mucositis (18 pts G2 and 5 pts G3-G4), FUO for 7 pts, 8 bacterial and 2 viral infection. 5 cases of atrial fibrillation were reported. One TRM in a pt unfit. The 5years-Cause Specific Survival (CSS) and the 5years-PFS of the series, calculated from ABMT were 86% and 78% respectively. During follow up 4 secondary tumors occurred (1 prostatic, 1 breast, 1 urothelial and one MDS).
Conclusions: The CGA was appropriate for selection of EP candidate to ABMT. It was superior to HTC-CI to reveal co-morbidity. Collection of CD34+, hematologic recovery, extra-hematologic toxicity were comparable to the younger pts. In the FIT EP, ABMT can be consider a safe treatment with similar results obtained for <60 years old pts. After ABMT follow up for second cancer is mandatory.

P872

Brazilian experience using high-dose sequential followed by autologous haematopoietic stem cell transplantation for malignant lymphomas

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High-dose cyclophosphamide (HDCY) followed by autologous stem cell transplantation (ASCT) is an effective and feasible therapy for refractory/relapsed lymphomas. In order to evaluate its use in a Brazilian population we analyzed all 106 patients (pts) with high-grade non-Hodgkin lymphoma (NHL) and 77 with Hodgkin Lymphomas (HL) submitted to this treatment between 1998 and 2006. High-dose sequential chemotherapy (HDS) consisted of the sequential administration of HDCY (4 or 7 g/m² dose), and granulocyte colony stimulating

factor (G-CSF) 300 µg/day, from day +1 after HDCY, followed by PBPC harvesting when white blood cells (WBC) increased to > 1.0 x 10^9/L, aiming to collect > 5 x 10^6 CD34+ cells/kg. At diagnosis, NHL patients had: median age 45 (8-65) years, 78% diffuse large B-cell lymphoma and 83% stage III/IV disease. HL patients had: median age 23 (7-68) years, 64.9% nodular sclerosis subtype, and 65% stage III/IV disease. As for toxicity, 9 (13%) HL and 10 (9%) NHL patients had grade I-II of cardiac toxicity. Forty (56%) HL and 47 (44%) NHL patients experienced grade I-III of gastrointestinal toxicity. Seven (9%) HL and 4 NHL patients (4%) developed acute renal failure not related to sepsis. Overall Survival for HL was 29%, disease free survival 59% and progression free survival 26%. NHL had 40%, 49% and 31% respectively. HDCY-related mortality was 10% for HL and 5% for NHL pts. HDCY dosing had no impact in toxicity or survival for both groups. Our study suggests this approach is efficient and feasible, regardless toxicity-related mortality.

P873

Mobilization of peripheral blood stem cells and autologous stem cell transplantation in HIV-related malignancies

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Background: Recent studies indicate that high dose chemotherapy (HDCT) followed by autologous peripheral blood stem cell transplantation (ASCT) may be effective in relapsed human immunodeficiency virus (HIV)-related lymphoma. However, further data are warranted.
Methods: Patients (pts) with HIV-related lymphoma or HIV-related germ cell tumor (GCT) were included in the ongoing cohort study. After mobilizing peripheral blood stem cells (PBSC) pts did or did not undergo consecutive ASCT.
Results: PBSC were successfully harvested in 10 of 11 HIV-infected pts with diffuse large B-cell lymphoma (DLBCL) [n=4], Burkitt's lymphoma (BL) [n=3], plasmablastic lymphoma (PL) [n=2], Hodgkin lymphoma (HL) [n=1] and testicular GCT [n=1]. The mean number of collected stem cells was 15.7x10^6/kg CD34+ cells (range, 6.3-33). PBSC-mobilisation failed in one pt with relapsed BL. So far, 5 of 10 pts received HDCT + ASCT. Pt 1 received HDCT as 3rd salvage therapy for DLBCL. A total of 9.2x10^6/kg CD34+ cells were transplanted, neutrophil engraftment occurred on day +14. The pt achieved a partial remission but died of progressive lymphoma 6 months after ASCT. Pt 2 underwent HDCT + ASCT (13.8x106/kg CD34+ cells) for a 1st relapse of HL. Neutrophil engraftment was observed on day +10. The pt is well and disease free 29 months after ASCT. Pt 3, a hepatitis C co-infected haemophiliac, received HDCT + ASCT for refractory DLBCL but died of liver cirrhosis and neutropenic sepsis with multi-organ failure on day +16. Pt 4 received 3 sequential courses of HD-carbopatin/etopside followed by ASCT in 3-week intervals for a 3rd relapse of a nonseminomatous GCT. Neutrophil engraftment occurred on day +10, +12 and +14, respectively. A complete remission (CR) was achieved but the pt suffered another relapse involving the central nervous system and died of progressive GCT 15 month after the 3rd transplant. Pt 5 underwent HDCT in 2nd complete remission after successful salvage-CT for a first sensitive relapse of DLBCL. A total of 12.9x10^6/kg CD34+ cells were transplanted. The pt died of candida pneumonia and multiorgan failure on day +42).
Conclusions: Successful mobilisation of PBSC is feasible in the majority of pts with HIV-related malignancies. ASCT is effective in selected cases of HIV-malignancies. However, treatment-related mortality appears higher than in the HIV-negative population.

P874

Impact of pre-transplant rituximab on clinical outcome after autologous haematopoietic stem cell transplantation in patients with CD20+ non-Hodgkin's lymphoma

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Introduction: The addition of rituximab (rtx) for the treatment of CD20+ Non Hodgkin's Lymphoma (NHL) is resulted in improvement of response rates and overall survival (OS).
Many patients (pts) develop refractory or recurrent disease also after that immunotherapy and the autologous hematopoietic stem cell transplantation (ASCT) remains the only therapeutic alternative.
Aim of the study was to evaluate the impact of pretransplant rtx on clinical outcome after ASCT in pts with CD20+ Non-Hodgkin's Lymphoma.
Methods: We analyzed 104 pts with Follicular, Diffuse Large B Cell or Mantle Cell Lymphoma, divided in two groups on the basis of pretransplant therapy with rtx (66 pts) or without rtx (44 pts), named R+ and R- pts, respectively. The two groups were similar for type of lymphoma, sex, lines of chemotherapy prior to ASCT, disease state at the transplant and conditioning regimen.
Results: The evaluation of the disease three months after ASCT showed a state of complete remission in 70% of R+ pts compared to 54% of R- pts.
We analyzed the OS and progression free survival (PFS) at 12 and 36 months.
At 12 months the OS for pts R+ was 88.7% vs 89.6% of R- pts (p=0.013); the PFS was 88.1% vs 89.1% (p=0.011) respectively.
At 36 months the OS for pts R+ was 70.2% vs 73.9% of R- pts (p=0.049); the PFS was 69.4% vs 73.3% (p=0.071) respectively.
The engraftment for absolute neutrophil count>500/microl occurred with a median time of 10 (r.9-16) and 11 (r.8-20) days for R+ and R- (p=0.003) pts, respectively; there was no statistically significant difference for platelet count>20000/microl with a median time of 13 days (r.9-23 R+ pts, 9-35 R- pts).
The period of febrile neutropenia was longer for R+ than R- pts (median time of 4 days vs 1 respectively; p=0.001). No significant difference was also documented in the incidence of CMV reactivation between the two groups (p=0.647).
The severity of mucositis was greater in R- pts with WHO 3-4 grade in 11.7% vs 20.4% in R+ vs R- pts, with a median time of 6.5 vs 5 days.
The transplant-related mortality was 3.3% for R+ pts vs 6.8% for R- pts (p=0.41).
Conclusions: These data showed that at short distance from the transplant, CR rate was greater in R+ pts than R- pts. At longest follow-up of 12 and 36 months, OS and PFS are better in R- group.
In R+ pts it was documented a more rapid engraftment and reduced severity of mucositis although febrile neutropenia was longer. There are no difference about platelet engraftment and CMV reactivation.

P875

Lymphocyte recovery and survival after autologous haematopoietic stem cell transplantation in refractory and relapsed Hodgkin's lymphoma

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Early lymphocyte recovery after autologous haematopoietic stem cell transplantation (ASCT) has been shown to be associated with positive clinical outcome in non-Hodgkin's lymphoma. However, there are conflicting data on whether early

lymphocyte recovery affects post-transplantation outcome in Hodgkin's lymphoma (HL). In this study we retrospectively identified variables affecting lymphocyte recovery and evaluated prognostic factors for the outcome of 93 patients (pts), median age 29 (16-59) years with refractory and relapsed HL treated with ASCT following a modified BEAM regimen (BCNU 300 mg/m^2, etoposid 800 mg/m^2, cytarabine 6000 mg/m^2, melphalan 140 mg/m^2, dex amethasone 168 mg/m^2). The indications for ASCT were: inadequate response to conventional therapy (52 pts), relapse (33 pts) and progressive disease (8 pts). The patients were treated with the median 2 (range 2-4) chemotherapy (chtx) lines before ASCT. Radiotherapy was performed in 45 pts prior to ASCT. The disease status at transplant was: CR (41 pts), PR (47 pts) and less then PR (5 pts). The source of progenitor cells was bone marrow (BM) (16 pts), peripheral blood (PB) (68 pts) and BM plus PB (9 pts). Results: The median follow-up was 53 (12-103) months. OS was 78% (95% CI 67-89) and PFS 71% (95% CI 60-82) at 5 yrs estimated with the Kaplan-Meier method. Prior radiotherapy was associated with lower absolute lymphocyte count (ALC) on day +15 after ASCT (p 0.015). Transplantation of BM as a source of progenitor cells was associated with higher ALC on day +15 comparing with PB (p 0.013). In univariate analysis, prognostic factors associated with decreased OS were the number of prior chtx lines (>2 vs 1-2 lines, p 0.0001), disease status at transplant (less then PR vs CR/PR, p 0.001) and ALC below 500/mm3 on day +15 (p 0.04). In multivariate analysis, the number of prior chtx lines remained statistically significant (HR 9.6, p 0.001) for OS. PFS was adversely affected by the number of prior chtx lines (>2 vs 1-2 lines, HR 7.3, p 0.0002) and the disease status at transplant (less then PR vs CR/PR, HR 1.5, p 0.08). We concluded that lymphocyte recovery after ACST is affected by prior radiotherapy and depends on the source of transplanted progenitor cells. Early lymphocyte recovery does not independently predict better survival after ASCT for HL pts. The outcome of pts with relapsed and refractory HL depends on the number of prior therapy lines and the disease status at ASCT.

P876

Cord blood transplantation in Waldenstrom macroglobulinaemia: report of two cases

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Allogeneic stem cell transplantation (alloSCT) in Waldenstrom's macroglobulinemia (WM) are considered in selected circumstances as salvage. Recent report of the french group SFGM-TC showed that alloSCT in refractory or relapsed WM led to an overall response rate of 92% with complete remission in 50% with a median follow up of 64 months (1). We report 2 cases of MW with a successfull unrelated cord blood transplantation (UCBT).
Cases 1: A 52 yo man, diagnosed in 1993 with IgM lambda level at 63 g/l. Physical examination was normal. He presented anemia (6.6 g/dl) and a bone marrow lymphoplasmacytic infiltration. Chlorambucil was continued until 2001 with a stable IgM level at 24 g/l; 1 year later anemia, thrombopenia and increased IgM level needed to start chemotherapy: 2 courses of fludarabine-cyclophosphamide, followed by R-CHOP because of pancytopenia. The persistant pancytopenia justified to performed UCBT in January 2005 with reduced intensity conditioning : fludarabine 200 mg/m², cyclophosphamide 50 mg/kg and TBI 2 Gy (TCF). He had no complication or adverse effects. Chimerism was full after 3 months. The IgM level was reduced from 10.5 g/l before UCBT to 4.3 g/l and the blood count was strictly normal. In November 2010, the patient was alive and well in very good partial response (2) with IgM component at 4,3 g/l. 2: A 56 yo man, diagnosed with WM in 1997. In 2004 he became anemic and bone marrow was infiltrated with 60% of lymphoplasmocytic cells. The IgM level was 18 g/l. Five courses of fludarabine and cyclophosphamide achieved a partial response with stable disease. In 2007, the blood count showed a pancytopenia with 70% of marrow lymphoplasmacytic cell infiltration. CHOP induced pancytopenia and severe pneumonia requiring intensive care. Nine courses of rituximab were then initiated until October 2008 with partial response and fluctuant severe cytopenia. Autologous collection was not possible and in absence of related or unrelated donor, UCBT was performed in November 2008 after TCF. Chimerism was full 3 months later with complete hematological recovery. In November 2010 he was alive and well in complete remission (2).
In conclusion, allogeneic SCT is a useful treatment option in advanced WM. These two observations and the reduced incidence of GVHD leads to considering UCBT for patients without related or unrelated donors.
(1) Kimby E et al. Clin. Lymphoma Myeloma 2006;
(2) Dhédin et al. Haematologica. 2010.

Multiple myeloma

P877

Stem cell transplant and zoledronic acid improve outcome in multiple myeloma

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High-dose chemotherapy following by stem cell transplant (SCT) after initial cytoreductive therapy is considered the treatment of choice in multiple myeloma (MM). Recently zoledronic acid (ZA) have been demonstrate antitumor activity in some human neoplasm, including MM. We assess if the use of ZA (4mg, standard dose, at monthly interval by 24 months) in untreated MM, whose were treated with DAI (dexamethasone, all transretinoic acid and interferon) and SCT can improve outcome measured by progression-free disease (PFD) and overall survival (OS). Three hundred and eigth patients were randomly assigned to receive ZA: 151 cases (arm 1) or not: 157 patients (arm 2). In an intention to treat basis, all patients were available for efficacy and toxicity. Overall response rate (ORR), complete response (CR) and Very good partial response (VGPR) were similar in both arms: 77%, 52% and 25% in ZA arm and 75%, 46% and 28% in control group, respectively. But, PFS and OS were better in ZA arm, PFD was 26 to 73 months (median 60.4) months in ZA compared to 16 to 46 (median 38.4 months) (p<.001) in control group. Actuarial curves at 5-years showed that OS was 67% in ZAarm and 48% in control arm (p<.001). Toxicity was mild, until now not jaw necrosis have been observed.
Conclusion: we can demonstrate that ZA have clinically antitumor activity in MM, and will be included in all patients with this neoplasm.

P878

Tumour-promoting properties of mesenchymal stem cells in multiple myeloma: a potential risk for therapeutical use after haematopoietic stem cell transplantation in myeloma patients?

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Mesenchymal stem cells (MSCs) have recently been used in several pre-clinical and clinical studies to support hematopoiesis after hematopoietic stem cell transplantation (HSCT), to control graft versus host disease after allogeneic HSCT or for gene therapy in cancer. Although it is believed that MSCs have therapeutical potential for these particular applications,

several studies have shown that MSCs might also play an active role in the pathogenesis and progression of tumors. Multiple myeloma (MM) is a malignancy of terminally differentiated plasma cells (PCs), which are predominantly localized in the bone marrow (BM). Mesenchymal stem cells (MSCs) give rise to most bone marrow stromal cells that interact with MM cells. However, the direct effect of MSCs on the growth control of MM cells has not been addressed. In the present study, we showed by in vitro migration assays that human MSCs are attracted by MM cells and that CCL25 is a major MM cell-produced chemoattractant stimulating chemotaxis of MSCs through CCR9. By coculture experiments we found that MSCs favor the proliferation of stroma-dependent MM cells through secretion of soluble factors and cell to cell contact. This growth promoting effect was also demonstrated by intrafemoral co-engraftment experiments in the in vivo mouse myeloma model 5T33MM. In addition we demonstrated that MSCs protect MM cells in vitro against spontaneous and Bortezomib-induced apoptosis. The tumor-promoting effects of MSCs correlates with their capacity to activate in MM cells AKT and ERK activities, accompanied with increased expresson of CyclinD2, CDK4 and Bcl-XL, and decreased cleaved caspase-3 and PARP expression. In turn, MM cells were found to upregulate interleukin-6 (IL-6), interleukin-10 (IL-10), insulin growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF) and dickkopf homolog 1 (DKK1) expression in MSCs. Finally, we demonstrated that intraveneous injection of in vitro expanded murine MSCs (mMSCs) in 5T33MM mice results in a significantly shorter survival as compared to the control group, being injected with MM cells without mMSCs ($p < 0.05$). Our data suggest that MSC-based cytotherapy has a potential risk to stimulate MM disease progression and/or relapse and should therefore be considered with caution in the treatment of MM patients after hematopoietic stem cell transplantation.

P879

The impact of the new anti-myeloma drugs on outcome of the allogeneic stem cell transplantation with reduced-intensity conditioning in patients with high-risk multiple myeloma

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During the last ten years the treatment of multiple myeloma has dramatically changed. The large utilisation of novel agents (lenalinomide, bortezomib) results in higher complete remission rates and longer overall and event free survival.
The purpose of this study was to evaluate the impact of these new drugs on the outcome high-risk multiple myeloma patients treated with RIC Allo-SCT in our program over the last 10 years; 45 patients (group1) transplanted before January 2006 and who had received neither of the novel agents prior to transplant was compared with 34 patients (group 2) transplanted after January 2006, who received either drugs or both before Allo-SCT.
The median time between diagnosis and Allo-SCT was 37 months (6-161) and 41 months (9-145) in the two groups respectively. The median follow-up after transplant was 45 (2-127) and 16 (3-39) months in the first and second group respectively. Age, sex, number of auto-transplantation, lines of treatment, stem cells source (peripheral stem cells, bone marrow), dose of CD34+/kg and CD3+/kg, conditioning regimen and post grafting immunosuppression (cyclosporine with or without mycophenolate mofetil) did not differ between the 2 groups. All vs. 21 patients (62%) had a match related donor in the first and in the second group respectively as compared with none vs.13 patients (38%) transplanted from an unrelated donor. No patient experienced a graft rejection.
The cumulative incidence of acute graft versus-host disease (GVHD) was significantly higher before 2006 (47% vs 24%; p=0.0584). The cumulative incidence of chronic GVHD was also different (56% vs 30%; p=0.0241).The estimated probability of non relapse mortality (NRM) at day 100 was 12% in the first group vs 0 % in the second group transplanted after 2006. The one and two years NRM was 18% vs 23% (p=0,537).
The overall survival (OS) at two years was 60% vs 70% in the first and second group respectively (p=0.1784). The progression-free survivals (PFS) was significantly different at 2 years, 45% before 2006 compared to 65% after 2006 (p=0,056).
Conclusion: In the era of novel drugs, we documented a lower incidence of acute GVHD and NRM, associated with a high CR rate; also the survival and relapse incidence were significantly different between the two groups. The development of novel reduced-intensity preparative regimens and peri- and post transplantation strategies enhancing the graft-versus-myeloma effect are the important key issues for the future.

P880

Lenalidomide as maintenance therapy in multiple myeloma after allogeneic stem cell transplantation induces strong T and NK cell response – Results from a dose-finding study

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A potential curative approach for pat. with multiple myeloma (MM) is allogeneic HSCT. HSCT with dose-reduced intensity conditioning is associated with a high number of relapses. There is a need of post transplant strategies to improve remission rates and disease free survival. Lenalidomide is an effective drug for pat. with MM. The immunmodulatory properties on T- and NK- cells of lenalidomide may augment the GvM effect after HSCT, but myelosuppression as well as induction of GvHD is a concern of using the drug early after HSCT. The aim of the study is to determine the max. tolerable dose of lenalidomide after HSCT. A total of 24 pat. were enrolled so far. Lenalidomide as maintenance treatment was started between 100 and 180 days after HSCT. In the first group 3 pat. started with dose of 5 mg/d day 1-21 for 4 cycles. In this group dose limited toxicities not appear. In the next higher dose level (10 mg/d) we treated a total of 6 pat. In this cohort 3 pat. showed DLT (acute pancreatitis, hepatotoxicity, attribute to liver GvHD, renal insufficiency and diarrhea grade III (GvHD)). Because 3 pat. in 10 mg cohort developed DLT, the maximum tolerate dose has been declared as 5 mg. So far 13 additional pat. were treated with this dose level (5 mg/d, day 1-21). In this cohort 4 pat. experienced CTC grade III-IV toxicity was observed (2 pat. with diarrhea (GvHD), hepatotoxicity, intolerance). We therefore, monitored T, B- and NK cell subsets by flow cytometry during lenalidomide treatment. We found a significant increase in activated T cells after two week of lenalidomide (13% vs 41%; $p<0.05$), this effect is mainly based on the activation of CD8 cells (29% vs 60%; $p< 0.05$). Interestingly we observed an increase in CD4/INFg as well as CD8/INFg cells in the first week after treatment (0.5% vs 6.5%; p=0.03 and 1.8% vs 6.7%; p=0.02 respectively). In contrast to those proinflammatory cells, regulatory subsets like Treg cells and B10 cells were found to decreased in the first week of lenalidomide (2.7% vs 0.5%; $p<0.05$ and 13% vs 6.5%; p=0.05 respectively). Along with the T cell data, NK cells expressed more activating receptors, like NKp44 and less inhibitory receptors, like NKG2A. This data highlight that lenalidomide has immune stimulatory properties and may contribute to the induction of GvHD. We concluded 5 mg lenalidomide is the maximum tolerate dose if used early after HSCT.

Lenalidomide has high immune modulatory properties that might increase the risk of GvHD.

P881

Efficacy, tolerance and immunomodulatory effects of lenalidomide as salvage treatment for multiple myeloma relapsing after allogeneic haematopoietic stem cell transplantation: a multi-centre retrospective study of the SFGM-TC

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Background: Lenalidomide is an efficient treatment of multiple myeloma (MM) with immunomodulatory properties that might be of particular interest in the context of allogeneic HSCT. In this study, we investigated the efficacy, tolerance and potential immunomodulatory effects of lenalidomide after allo-HSCT.
Patients: This multicentric retrospective study from 13 French centres included 54 MM patients having received lenalidomide as a salvage therapy after allo-HSCT. Median age at transplant was 51 years (range: 32-61). Allo-HSCT was performed in heavily pretreated MM patients, mainly with a reduced intensity conditioning (87%) and PBSC (86%) from a sibling donor (88%). Lenalidomide treatment started at a median time of 24 months (range: 1-97) after allo-HSCT at the dose of 25 mg/day in association with dexamethasone in 76% and 78% of cases, respectively.
Results: Patients received a median of 6 cycles of lenalidomide (range 0,2-23). Overall response rate was 79 %, (28% CR, 22% VGPR, 29% PR). Usual haematological (29% neutropenia, 13% thrombocytopenia), infectious (43%), gastrointestinal (19%) and neurological (26%) side effects were observed. Most of those (94%) were reversible with only one death attributed to treatment toxicity. During lenalidomide therapy, 17 pts (32%) experienced de novo acute GVHD (aGVHD) with 6≥ grade 3 aGVHD, all of those had been controlled by standard immunosuppressive therapy and/or lenalidomide withdrawal. Only 4 new cases of chronic GVHD were reported after the introduction of lenalidomide. With a median follow up of 16.9 months, the median overall survival (OS) was not reached (estimated 2 years OS= 66%) and the median progression free survival (PFS) was of 18 months. Median time to best response to lenalidomide was shorter in patients who experienced aGVHD under treatment (5,1 vs 2,4 months, p=0,001). Among non refractory patients (n=50), the use of a maintenance treatment with lenalidomide (n=20) had no significant impact on PFS and OS (p=0.11 and 0.12, respectively). Relapse under lenalidomide treatment was associated with an impaired OS (p=0.0379). Peripheral T cell recovery analyzed by flow cytometry in 12 pts before and after lenalidomide treatment showed relative increases in CD8+ T cells while CD4+ T lymphocytes remained globaly stable.
In conclusion, lenalidomide appears as an efficient and safe salvage treatment option after allo-HSCT with potential immunomodulatory effects in vivo.

P882

Prognostic factors and outcome of reduced-intensity conditioning followed by allogeneic stem cell transplantation in patients with multiple myeloma relapsed after autografting and rescued with new drugs

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Background and objectives: The use of allogeneic stem cell transplantation (allo-SCT) with reduced intensity conditioning (RIC) in relapsed multiple myeloma (MM) is controversial due to the mortality and the morbidity related to the procedure and the availability of novel antimyeloma drugs. We investigated prognostic factors and outcome of RIC allo-SCT in MM patients who relapsed after autologous SCT and were then treated with a salvage therapy based on novel agents.
Patients and methods: Sixty-eight patients, median age 53 years, were retrospectively evaluated in a multicenter study. Median time between auto-SCT and relapse was 15 months (2-87). Salvage treatment was thalidomide-based (38 patients), bortezomib-based (24), lenalidomide-based (6), and achieved 23 complete remissions (CR) + very good partial remission (VGPR) (33%) and 32 partial remissions (PR) (47%). Twenty-four (35%) had an HLA identical sibling donor and 44 (65%) had an unrelated donor. Fifty-seven patients (84%) received peripheral stem cells. Most used preparative regimens consisted of fludarabine, melphalan ± thiotepa (28 patients) and fludarabine + 2 Gy TBI (24 cases).
Results: Two-year cumulative incidence of non-relapse-mortality (NMR) was 22%. Two-year progression-free-survival (PFS) and 2-year overall survival (OS) were 37%, and 52%, respectively. Grade II-IV acute GVHD and chronic GVHD occurred in 28 (41%) and 21 (39%) evaluable patients, respectively. Thirty patients (44%) showed relapse or progression and received one or more new drugs (23 patients) ± donor lymphocyte infusions (DLI) (9 patients). The observed median OS after relapse post-allo-SCT was 4,5 months. At a median follow-up of 29 months after allo-SCT, 27 patients (40%) maintained a clinical objective response, 9 of them achieved again a response to new drugs ± DLI after they had progressed post allo-SCT.
In multivariate analysis chronic GVHD significantly prolonged OS (HR 0.11; 95% CI, 0.17-0.68, p=0.02), whereas a longer time between diagnosis and allo-SCT was significantly associated with poor OS (HR 1.07; 95% CI, 1.01-1.13, p=0.02).
Conclusions: These results suggest that an earlier timing of allo-SCT and new transplant strategies that will incorporate novel agents before and after allo-SCT could improve clinical results of RIC allo-SCT in relapsed MM.

P883

Allogeneic HSCT in multiple myeloma – Double ASCT and RIC as risk factors

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The outcome of patients with multiple myeloma is still unsatisfying. Although recent years saw survival improvement, most likely due to high dose chemotherapy and autologeous SCT (ASCT), disease progression just seems to be deferred, eventually leading to heavily pretreated patients whose survival will depend on not yet established drugs or regimen.
Allogeneic human stem cell transplantation (HSCT), due to myeloma free graft and the graft versus myeloma effect, bears the potential of cure, however, high TRM hampered this approach.

Reduced intensity regimens (RIC) lead to a drop in TRM, just to be offset by higher relaps rates. Hence, no consistent superiority in OS/ PFS was seen for RIC HSCT when compared to ASCT in three recently published prospective studies.
We provide the result of a retrospective, single center analysis of 78 patients receiving allo- HSCT for multiple myloma between 1994 and 2009. Median age was 49 years, 24 patients had received more than one prior ASCT, 32 patients had SD (n=5) or even PD (n=27) at time of HSCT. Matched related donors were available in only 32 cases. Preparative regimen consisted of either Melphalan (once 140 mg/m^2 or twice 100 mg/m^2), TBI (8-12Gy) based or reduced intensity regimen (n=26), usually in conjunction with Cyclophosphamide, Fludarabin and ATG. After HSCT a CR/ VGPR was reached in 93% of all evaluable patients. Five patients died prior to staging, the 2y NRM was 19,2% (total 21,8%). Chronic GvHD was seen in ~25%.
Risk factors associated with diminished PFS and OS (given data) were >1 prior ASCT (HR 3,39, p=0,00), allo-HSCT more than 10 month after last ASCT (HR 1,95, p= 0,03), no PR at time of HSCT (HR 2,57, p 0,002), use of a "RIC" regimen (HR 2,14, p=0,01) and a lower CD34/MNC count (HR 2,05, p=0,38) in univariate analysis. In multivariate analysis >1 prior ASCT and "RIC" could be confirmed as independent risk factors. Regarding the latter risk factors, patients in at least PR prior to HSCT reached an survival plateau at >63% (n=30).
Summarizing the results, we established >1 prior ASCT as an independent risk factor for subsequent HSCT, which might be of importance especially in treatment of younger patients. We saw good responses with moderate TRM rates after "full intensity conditioning" HSCT, resulting in a significant better outcome compared to our RIC regimen. This data may imply, that earlier and more aggressive treatment has the potential to improve the still unsatisfying outcome in multiple myeloma.

P884

Introduction of differential KIR activation mediated patterns of NK cell cytotoxicity against myeloma

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Natural Killer cells (NK cells) have the ability to recognise and kill malignant cells, like myeloma cells. Killer immunoglobulin like receptors (KIR) are a family of receptors that has activating and inhibitory function on NK cells. To clarify how KIRs are involved in MM cell cytotoxicity we investigated 3 different model systems for NK cell alloreactivity:
1) HLA-C/ HLA-C interaction model (KIR-Ligand model),
2) HLA-C/ KIR receptor interaction model, and 3) impact of donor KIR haplotype.

Different myeloma cell lines having a C1/C1, C1/C2 or C2/C1 HLA-C background and a NK cell line (NKL) were used for the experiments. NKL cells were transfected with human KIR2DL1 and KIR2DL3 cDNA, respectively. NK cells from healthy donors were typed for expression of HLA-C molecules, KIR receptor expression or KIR haplotype. Thereafter NK cells were transiently transfected with the siRNA or control siRNA against the KIR2DL1 or KIR2DL3 receptors. Functional analysis of the NK cell cytotoxicity was measured by their killing ability against the fore mentioned myeloma cell lines.
Using NK cells that have been genotyped as HLA-C1/C1, we observed a rescue of C1/C1 positive meyeloma cells in contrast to C2/C1 myeloma cells (12% cytotoxicity vs. 38% and 45% cytotoxicity, respectively, p <0.05). We used NK cells from healthy donors, which express KIR2DL1 (recognizing C2) to evaluate whether receptors RNAi knockdown could increase the ability of NK cells to lyse target cells. Knockdown of KIR2DL1 in NK cells results in a highly efficient killing of a C2/C2 target cell. To evaluate whether KIR receptors are major inhibitory molecules, we used a NK cell line (wt NKL, a non expressor of KIR receptors) with high cytotoxic potential against all tested target cell lines. This NK cell line was transfected with KIR2DL1 or KIR2DL3. C2 positive myeloma cell lines were significantly rescued when exposed to KIR2DL1 transfected NK cells (wt NKL 72% vs. KIR2DL1 NKL 29%, p <0.05). Similar results were obtained when C1 positive target cell line was co- cultured with KIR2DL3 transfected NK cells. Testing the KIR haplotype model, we found that NK cells with an AA KIR haplotype showed the lowest killing capacity, while BB KIR haplotype had higher killing potential. (46% vs 59%, p=0,005) against target cells.
We have shown that NK cell cytotoxicity is significant regulated by all 3 tested models. These data underline the importance of KIR receptor modulation on NK cell reactivity against myeloma cells.

P885

Bortezomib-including mobilization and conditioning is feasible in elderly multiple myeloma patients and can achieve negativization of the minimal residual disease

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Introduction: Autologous Stem Cell Transplantation (ASCT) is part of the treatment strategy in eligible patients (pts) with MM. High-dose melphalan (HDM:200 mg/m^2) is the recommended conditioning before ASCT; synergistic effects of Bortezomib (BOR) and HDM are reported in vitro and in vivo. We started a phase 2 trial in MM elderly patients, fit for ASCT, combining BOR, CY and dexamethasone (DEX) as induction and mobilizing therapy (CY-BOR), followed by ASCT with BOR-HD-MEL to evaluate the feasibility (safety and RRs) of this approach.
Patients and methods: We enrolled 30 pts (17F/13M, median age: 65 yrs, range 52-77). Pts in at least PR after 4 CY-BOR courses were mobilized with BOR and DEX standard schedule with CY 3g/m^2 (day 8). Pts collecting at least 2.5x10^6 CD34+/kg underwent ASCT with HD-MEL (day-1) and BOR (1 mg/m^2 on -6,-3,+1,+4), followed by thalidomide consolidation until Relapse/Progression. The percentage of plasma cells (PCs) in PBSC harvested and in bone marrow along different steps of therapy was assessed by 4 colour flow cytometry (FC) with CD38, CD45, CD56, CD138, CD19, CD27, CD28, CD117, kappa and lambda staining. DNAs were stored to evaluate MRD (with patient-specific probes) in the harvest and in vivo after ASCT.
Results: Of 30 pts evaluable for response before ASCT, 22 (73%) achieved at least PR and 20 (67%) were mobilized: 19 (63%) of them were able to mobilize ≥2.5x10^{6}CD34+/kg and 18 underwent ASCT. Median time for PMN engraftment was 11 days (range 10-13) and 14 (range 12-20) for PLT≥20.000/mcl. We did not observe any grade 3/4 infections; 2 pts experienced a grade 4 neurologic toxicity. With a minimum follow up of 167 days (median 590, range 167-956) 17/18 transplanted pts are alive: one died of Disease Progression. At day +90 after ASCT 16 pts are evaluable for response: all are responders (11 CR+nCR; 5 VGPR); 9 improved their response after ASCT. Fifteen patients were evaluated by Flow Cytometry in order to detect clonal plasmacells (cPCs) along several steps of treatment: 3/15 pts achieved MRD clearance at day +90 (cPC <0.01%); only 1 pt achieved MRD negativity before ASCT. Flow cytometry evaluation of cPCs in the harvest from 13 patients, showed that the cPCs were <0.01% in 11 pts.
Conclusions: ASCT with HDM and BOR is feasible in older patients, with very high RRs and without major toxicities. We need a longer follow up and a larger number of pts to assess if these results will translate in a benefit in terms of outcome.

P886

Fludarabine and busulfan x 4 conditioning prior to allogeneic haematopoietic cell transplant for high-risk multiple myeloma

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Multiple myeloma (MM) is an incurable disease. Targeted chemo-biologic therapy and autologous hematopoietic cell transplant (HCT) are largely ineffective in controlling aggressive MM. Although allogeneic HCT has the potential to cure the disease, the outcomes remain poor due to high treatment-related mortality (TRM) from myeloablative conditioning, increased relapse from non-myeloablative regimens and possibly other issues that are specific to this patient population and are less well understood. We explored feasibility and efficacy of a myeloablative regimen, with fludarabine and busulfan (FluBu4) [fludarabine 40 mg/m²/d and busulfan 3.2 mg/kg/d IV x 4 days] in patients (pts) with high-risk MM. High-risk disease was defined by unfavorable cytogenetics [t(4;14), t(14;16), t(14;20), -13, -17/17p, hypodiploidy] or early relapse following autologous HCT. Since 2008, a total of 17 pts have been enrolled. The majority (n=10, 58%) had prior autologous HCT, with a median *HCT-specific comorbidity index (HCT-CI) of 3 (range, 0-6). Donor types included: 8 matched related, 5 matched unrelated and 4 mismatched unrelated. Graft-versus-host disease (GvHD) prophylaxis included tacrolimus/methotrexate in 15 (88%) and tacrolimus/mycophenolate in 1 (6%). Pt characteristics and MM status are shown in Table 1. All 17 pts tolerated the conditioning well, without early toxic deaths or graft failure. Common regimen related toxicities include mucositis (n=17, 100%) and mild transient liver function abnormality (n=9, 53%). Grade 3-4 mucositis occurred in 5 pts (29%). Twelve pts (71%) developed acute GvHD, and this was grade II-IV in 7 (41%) and grade III-IV in 4 (23%). Chronic GvHD occurred in 10 pts (59%). With a median follow-up of 238 (range, 23-899) days, the median overall survival has not reached; thirteen pts (76%) are still alive and 10 of them are without disease. Six pts (35%) had relapse of their myeloma, and 3 of them died. Only 1 pt without relapse had a transplant-related death due to idiopathic pneumonia syndrome. This pt had marginal pulmonary function pre-HCT, with HCT-CI of 3. There were no deaths related to GvHD; thus TRM was 6% only. The median progression-free survival was 373 days (95% CI, 50-695). In summary, myeloablative HCT using FluBu4 conditioning for high-risk MM appears to be a feasible and promising platform to combine with newer therapeutic options for pts with high-risk MM.

Table 1.

Variables	N = 17
Sex : Male/Female	11 : 6
Median Age at Transplant in Years (range)	54 (47-70)
Myeloma Status at Transplant	
PR1 / VGPR1	3 / 1
PR2 / VGPR2 / SD2	6 / 4 / 1
PR3	1
PR4	1
Prior Autologous Transplant	
0	7 (41%)
1	8 (47%)
2	2 (12%)
***HCT-CI** (median, range)	3 (0-6)
Karnofsky Performance Status	
>80%	11 (65%)
≤80%	6 (35%)
Donor	
HLA-MRD/-MUD/-mismatched UD	8 / 5 / 4
Median Cell Dose [x10⁶ CD34+cells/kg] (range)	6.3 (4.4-10.7)
Median Time to Engraftment in Days (range)	
Neutrophil	11 (10-14)
Platelet	11 (0-15)
Regimen Related Toxicities	
Gastrointestinal: Mucositis	17 (100%)
Grade 1-2	12 (70%)
Grade 3-4	5 (30%)
Hepatobiliary: Grade 1-2	9 (53%)
Infection: Grade 1-2	3 (17%)
Pulmonary (Idiopathic pneumonia syndrome): Grade 5	1 (6%)
Acute GvHD	12 (71%)
Overall Maximal Grade I / II / III / IV	5 / 3 / 1 / 3
Chronic GvHD	10 (58%)
Limited Type	3 (18%)
Extensive Type	7 (41%)
Median Follow-up Time in Days (range)	238 (23-899)
Median Overall Survival in Days (range)	Not Reached
Median Progression Free Survival in Days (range)	373 (50-695)

*Sorror ML, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood 2005;106:2912-19.

P887

15 years of single-centre experience with stem cell transplantation for multiple myeloma

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Introduction: Autologous stem cell transplantation (ASCT) became standard of care for patients with multiple myeloma (MM) under the age of 65 years. We routinely perform ASCT for newly diagnosed MM since 1996 in our department.
Patients and methods: We retrospectively analyzed all 286 transplants in 186 patients done for MM from 1996 till 2010. 271 transplants were autologous (254x Mel200, 17x Mel100 for elderly patiens or as salvage regimen), 15 transplants were allogenic (13x reduced-intensity conditioning Bu-Flu-ATG or Flu-Cy, 1x myeloablative Bu-Mel, 1x twin sibling). Median age of patients was 58 years (27-75). We analyzed overall survival (OS) and progression-free survival (PFS) regarding conditioning, stage, complete or very good partial remission (CR, VGPR) achievement, renal impairment, single vs. double transplant and use of new drugs (thalidomide and bortezomib).
Results: Estimated 15-years survival of the whole set of patients is nearly 40%. Patients who underwent double auto-allogenic transplantation as primotherapy had significantly lower PFS (p= 0.001) and also OS (p=0.018) compared to those who had only ASCT. Patients with renal impairment had significantly shorter OS than those without (p=0.03). Patients with Mel100 conditioning had trend towards worse OS than those with Mel200, however statistical significance was borderline. We observed trend towards better outcome in patients treated with new drugs. Reaching CR or VGPR was surprisingly not translated into better OS and PFS. Also stage of the disease and whether single or double transplant was used did not make any significant difference in the outcome.
Conclusion: Stem cell transplantation greatly improved outcome of patients with MM. Poor outcome of allogenic transplantation in our group of patients is related to high transplant related mortality (20% vs. 0.3%) and unexpected high relapse rate. However in this group are two long term survivors who are disease free for more than 10 years. There is an obvious trend towards better survival, when new drugs are incorporated at any time in the course of the disease as an addition to ASCT. This fact supports hypothesis that use of these drugs before ASCT could further improve long-term outcome in all patiens with MM. Our results also suggest that Mel100 regimen is insufficient and other treatment options should be offered to older patients. This is in good agreement with results of recent clinical trials.
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P888

Multiple myeloma and severe renal impairment: a safe preparative regimen proposal for autologous stem cell transplant

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Autologous stem cell transplantation (ASCT) is a well established procedure to consolidate remission in patients with multiple myeloma (MM); current guidelines recommend the use of Melphalan given as a single agent at the dosage of 200 mg/m² in patients up to 65 years old. The presence of comorbidities like cardiac disfunction, neuropathy or renal impairment require

dose reduction to lower the risk of therapy-related toxicity. In our institution patients MM and renal impairment, defined as a stable serum creatinine level above 1.5 mg/dl, receive melphalan at the dose of 160 mg/m^2; patients already on hemodyalisis undergo the procedure before and four hours after melphalan infusion, while the ones with renal failure but dyalisis free receive the drug in a hyperhydration preparative regimen. Haemopoietic stem cell support is cleared of DMSO (dimetilsulfossido) and given 36 hours (day 0) after melphalan infusion.
From 2003 to 2010 256 patients with MM and amyloidosis underwent ASCT in our division; eleven of them had renal impairment (8 MM, 2 Amyloidosis, 1 Crystalcryoglobulinemia): seven were males, four females, median age was 50 years (range 30-65), median creatinine value was 4 mg/dl (range 1.5-9), median creatinine clearance was 20 ml/min (range 9-62). Status of disease at transplant was complete remission in one patient, near complete remission (nCR) in two patients, very good partial remission (VGPR) in one patient, partial remission (PR) in four patients, while three patients had active disease. Median Melphalan dose was 140 mg/mq (range 90-200), administered at day -2 and followed by autologous peripheral stem cells infusion at day 0 after DMSO removal (median number of CD34+: 5 x 10^6 cells/Kg, range 4-8). All patients engrafted, with a median time to engraftment of 12 days (range 10-21). Median duration of hospitalization was 23 days (range 19-31). We observed grade II oral mucositis in three patients, one ARDS-like pulmonary, one severe gut mucositis. With a median follow up of 26 (range 1- 60) nine patients are alive, two died for progression of disease; seven patients achieved a favourable response (4 CR, 1 VGPR, 2 PR), two have stable disease. Of the six people on hemodyalisis one died of disease progression and one became hemodyalisis free after transplant.
These data suggest that autologous stem cell transplant is feasible and safe in this subset of patients and that dose reduction doesn't jeopardize its efficacy.

P889
Efficacy and outcome of allogeneic transplantation in rare myeloma
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The rare Myelomas, IgD, IgE and IgM (but not non-secretory, NS) are known to have a poor prognosis both with conventional therapy and autologous transplantation. Little is known about the impact of allogenic transplantation in these conditions.
Using the EBMT database (Med A and Med B) we have conducted a retrospective study of 1437 patients with myeloma who underwent allogenic transplantation between 1985 and 2009 with complete data for age, sex and type of myeloma.
There were 795 IgG, 275 IgA and 285 bence jones (BJ) myelomas (common myelomas) with 26 IgD, 5 IgM and 52 NS with no IgE myelomas. There was no difference between the groups, common IgD/IgM NS in the age, Sex, Albumin, Calcium, Creatinine, HB and Salmon Durie stage at diagnosis. Time to transplantation was similar in all groups as was the proportion of patients having standard myeloablative or reduced intensity conditioning and T cell depletion or use of TBI. Serum β2M was higher in the IgD/IgM groups (P=0.011). The rare myelomas had a better response to initial therapy; the proportion of CR in the 3 groups was common; 16.3% IgD/IgM; 28% and NS; 42.5% (P=0.01). Overall survival and progression free-survival appeared worse in the rare myelomas: common myeloma; 30.6/15.6 months, IgD/IgM; 16.2/16.2 months and NS; 45.0 and 14.9 months respectively. Due to crossing of the curves the significance of these differences were difficult to interpret, but some tests showed that the IgD/IgM group were significantly worse than the other groups. There was no difference between the groups with respect to engraftment, transplant related mortality, relapse and graft versus host disease.
Further studies will be undertaken in patients having a standard (myeloablative) or RIC conditioning but numbers in the rare groups are small.

P890
Prognosis of multiple myeloma patients after autologous stem cell transplantation in the last decade. Comparison of two cohorts with different induction treatment approaches
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Background: Bortezomib or IMID-based induction regimens have improved pre-SCT overall and complete remission (CR) rates in MM patients. However, post-SCT results and disease-free survival (DFS) are only marginally superior to those obtained with dexamethasone-based inductions. In available randomized trials, overall survival (OS) has not substancially changed regardless of the first line pre-SCT induction administered.
Patients and methods: Data of two cohorts of patients with a newly diagnosed MM, treated in a single centre and submitted to ASCT in first response were collected. The first cohort (C1: 1999-2005) received dexamethasone and anthracyclin-based induction before ASCT and bortezomib and/or IMIDs at relapse. The second cohort (C2: 2005-2009) received IMIDs and/or bortezomib first line followed by ASCT and were treated at relapse with the same or alternative drugs. All patients had at least one year follow-up after ASCT. Post-ASCT CR rates, time to progression (TTP), event-free survival (EFS), time to next treatment (TNT) and OS were compared for both cohorts.
Results: Out of 141 potential ASCT candidates diagnosed during both periods, 88 received an ASCT after induction (N=49 in C1, N=39 in C2). Their median age was 58 years (range 32-68). Both cohorts were comparable in terms of gender, age, type of myeloma and stage. Median time from diagnosis to ASCT was 34 weeks (range 14-80 weeks). Post-SCT CR rates were 35% for C1 and 61% for C2 (p=0.025). During the first year post-ASCT 6 patients died due to toxicity or infection (12%), 3 relapsed (6%) and 1 died of unrelated causes among 49 patients at risk in C1. During the same period, 1 patient died due to progression (2%) among 39 patients at risk in C2. Both the probability of death of any cause (p=0.012) and the probability of infectious or toxic death (p=0.025) were significantly higher for C1 during 1-yr post-SCT.
After a median follow-up of 6 years for C1 and 3 years for C2, outcome endpoints are shown in the table.
Conclusion: In our experience, patients with MM who received chemotherapy with dexamethasone and anthracyclins as first line induction presented an increased risk of death during the first year post-ASCT compared with those receiving bortezomib or thalidomide. Post-ASCT CR rates and survival were superior for patients treated with bortezomib or thalidomide as induction.
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	Cohort 1		Cohort 2		
	Median (years)	95%CI	Median (years)	95%CI	p-value
TTP	2	1.5-2.7	3.4	1.8-5	0.243
EFS	1.8	1.1-2.4	3.4	1.8-5.1	0.049
TNT	1.9	1.3-2.4	3.6	2.3-4.9	0.034
OS	4.1	1.1-7	NA*	NA*	0.02
*projected post-ASCT for C2 at 4 years was 83% (95%CI 68-98%).					

P891

Allo-HSCT enhances the therapeutic effect of lenalidomide

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Lenalidomide (Len), a highly effective drug against multiple myeloma (MM), acts through several mechanisms, such as a direct cytotoxic effect, anti-angiogenesis, microenvironment modifications, and immunomodulation. The latter property is particularly interesting in the allogeneic hematopoietic stem cell transplantation (Allo-HSCT) setting, since Len may interact favourably with the graft-versus-myeloma (GVM) effect. Preliminary results from retrospective studies on heterogeneous patient populations have suggested that Len is more effective when given after Allo-HSCT. In order to verify this observation, we are conducting a case-matched analysis of Len after autologous stem cell transplantation (Auto-HSCT) or Allo-HSCT. The hypothesis is that Len may be more potent when administered after Allo-HSCT. In this retrospective study the matching criteria was represented by the number of treatment lines received before Len. In an attempt to uniform the treatment regimens, an intra-centre matching was required. To November 2010 we collected data from 20 patients in each group. Baseline characteristics between Auto and Allo patients were similar, except for age at diagnosis (57 years, range 45-70, in Auto patients; 47 years, range 29-55, in Allo patients; p=0.0005). The median number of previous lines of treatment was 3 (range 1-6) for both groups. Eighteen out 20 Allo patients received Allo-HSCT after the first line. Sixteen (80%) Auto and 19 (95%) Allo patients received bortezomib in previous lines. Similarly, 14 (70%) Auto and 12 (60%) Allo patients were previously treated with thalidomide. Len was always combined with dexamethasone. Best responses were for Auto and Allo patients as follows: 2 vs. 2 CR, 4 vs. 7 VGPR, 3 vs. 4 PR, 3 vs. 3 PR, 7 vs. 4 PD. Time to the best response was 2.5 months (range 1-6) for Auto, and 3.5 months (range 1-19) for Allo patients. One year and 2 year progression-free survival were 28% and 9% for Auto patients, and 61% and 61% for Allo patients (p=0.03), respectively. Two years overall survival was 55% for Auto and 89% for Allo patients (p=0.03). Similar results were observed regardless of previous thalidomide treatment. No unexpected toxicities were reported. Two (10%) patients had a worsening of a pre-existent extensive chronic GVHD.

In conclusion, the preliminary data of our study support the hypothesis that Len is synergistic with the GVM effect, still retaining a favorable toxicity profile.

P892

Inhibitory KIR 2DL5 influences the outcome of autologous haematopoietic stem cell transplantation for multiple myeloma

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Autologous hematopoietic stem cell transplantation (AHSCT) is recommended for the treatment of patients with active multiple myeloma who are under 65 years of age and do not suffer from other serious comorbidities. However, although this therapeutic approach remains the only potential cure, not all patients achieve complete remission. Indeed, about 40% of treated patients become resistant to chemotherapy and present with relapse following AHSCT. Clearly the patient's response to chemotherapy is influenced by a number of factors, capable of modifying the outcome of the transplant procedure. It is not unlikely that immunogenetic factors play a role, particularly the reactivity of natural killer (NK) cells and polymorphisms of killer cell immunoglobulin-like receptor (KIR) genes. The present study was designed to investigate the possibility of a relationship between KIRs and the outcome of AHSCT.

Fifty-two patients received AHSCT for multiple myeloma in our Center from April 2003 to October 2010. Induction therapy consisted of thalidomide-dexamethasone (32 subjects) combined with bortezomib (20 subjects). Both KIRs and their respective HLA Class I ligands were studied in all recruited subjects. The KIR gene frequencies of our patients overlapped well with those of the general population which was represented by 181 healthy individuals of the Sardinian Voluntary Bone Marrow Donor Registry.

Twenty-eight patients (54%) achieved complete remission (CR) or a very good partial response (VGPR). The remaining 24 patients (46%) only achieved a partial response (PR) and/or relapsed (R).

A statistically significant difference was observed between these two groups of patients for the 2DL5 inhibitory KIR gene, KIR 2DL5 [CR/VGPR = 35.7% vs PR/R = 83.3%, hazard risk (HR) = 0.24, 95% confidence interval (CI) 1.3 – 58.4, P =.02].

Patients carrying only 5 of the 7 possible KIR genes responsible for inhibitory functions had a four-fold lower risk for developing relapse (HR=0.24). If our data are confirmed, then this parameter may become a useful tool for the identification of patients at high risk for relapse who require careful monitoring during AHSCT.

P893

Melphalan 100 mg/m² with stem cell support as first-relapse treatment is safe and effective for myeloma patients with long remission after autologous stem cell transplantation

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Introduction: High-dose melphalan (200 mg/m^2) followed by autologous stem cell transplantation (ASCT) is stille the backbone of standard therapy for younger patients. Yet, multiple myeloma is a chronic disease and the vast majority of patients relapse after primary treatment. For patients in first relapse there is no consensus on which regimen should be used, sequencing therapy or multidrog combinations, which is reflected by the lack of randomised trials adressing this question. However, the fact that many patients experience a good and durable response to primary ASCT raises the important question whether to retreat with the same regimen or to introduce a new line of therapy. If patients retain sensitivity to previous treatment at relapse, other agents could be reserved for later use. A second ASCT could however result in considerable toxicity in this setting and lower doses of melphalan might be preferable.

Aims: To evaluate the response rate, toxicity and survival (also in relation to response duration of first ASCT) for patients in first systemic relapse treated with melphalan 100 mg/m^2 and stem cell support (MEL 100).

Results: Sixty-six patients, with a median age of 61 years, were treated with MEL 100. The overall response was 79% (23% VGPR, 56% PR) and no treatment-related mortality was observed. There was limited, mostly hematological toxicity, and many patients could be treated in an out-patient fashion. The median stay in hospital the first 30 days was 3 (range 0-90). With a median follow-up of 23.8 months (range 3.5-86.9), the

median progression free survival (PFS) was 8.5 months and the median overall survival was 24.1 months. A regression analysis of the correlation between first and second remission suggests that patients with a PFS of 22 months or more in first remission had most benefit from MEL 100: median PFS was then 10 months.
Conclusion: We suggest that, when autologous stem cells are collected up-front, one should be aiming at a surplus. MEL 100 could then be a possible treatment alternative at relapse, especially for patients who had a durable response after first ASCT.

P894
Myeloma cell contamination of peripheral blood stem cell harvests estimated by multiparametric flow cytometry: potential correlation with disease outcome
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High-dose therapy and autologous stem cell transplantation (ASCT) remains an integral component of the myeloma treatment algorithm for patients considered eligible for the procedure. Recent studies suggest that the presence of malignant plasma cells in the peripheral blood stem cell harvest (PBSCH) may correlate with the clinical outcome after high dose chemotherapy followed by ASCT.
Objectives: To estimate the presence and the proportion of plasma cells (PC) in PBSCHs in correlation with clinical data.
Methods: PBSCHs from 30 myeloma patients (21 men and 9 women, n=30) who underwent ASCT were studied by 6-color flow cytometry (FCM) using the following combination of fluorochrome-conjugated antibodies, CD38/CD56/CD45/CD117/CD138/CD19. Response to therapy was assessed at the time of mobilization, at day +100 and at the end of the study after 14 months median period of follow up. The 2006 International Myeloma Working Group criteria were applied as follows: complete response (CR) (n= 8), very good partial response (VGPR) (n=8), and partial response (PR) (n=14). Mobilization regimens included G-CSF (n=30), Cyclophosphamide (n=3), Plerixafor (n=2). The Chi-square and Fisher exact tests were used to compare differences between nominal variables and the Mann-Whitney U test for continuous variables.
Results: Plasma cells bearing CD138+CD38+CD45+CD19+CD117-CD56- immunophenotype were identified in 29/30 cases. The mean PC percentage by FCM was 0,029% (0-0,27%). Plasma cell contamination in PBSCHs showed positive correlation with (1) the degree of bone marrow infiltration by morphology (Pearson correlation 0,62; p=0,000) and (2) monoclonal immunoglobulin levels (Pearson correlation 0,4; p=0,043) at mobilization. Importantly, patients who achieved CR at day 100 had ten-fold lower PC in the PBSCH than those with VGPR or PR (mean 0,005% vs. 0,046% Mann Whitney U, p=0,02). Moreover, at latest follow-up, patients in CR showed significantly lower levels of contamination compared to the VGPR/PR group (mean 0,0046% vs. 0,03%, p=0,006).

P895
Allogeneic stem cell transplantation in multiple myeloma
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Background: Allogeneic stem-cell transplantation (allo-SCT) is a potentially curative therapeutic approach for patients with multiple myeloma (MM). However, the role of allo-SCT is controversial due to the high attributable procedure-related mortality previously reported.
Objective: To analyze morbidity and mortality of patients with MM undergoing allo-HSCT. Patients and method: during the last three years, 20 patients (12 male, and 8 female) with MM underwent allo-SCT in our institution. Conditioning regimen was with fludarabine (150 mg/m^2), melphalan (140 mg/m^2) and thymoglobulin (1.5-3 mg/kg) in 80% of the patients. Prophylaxis for graft versus host disease (GVHD) consisted of cyclosporin A plus short methotrexate (x4). All patients were nursed in single rooms with high-efficiency particulate air filter. Antibiotic prophylaxis consisted of trimethoprim-sulfamethoxazole, ciprofloxacin, fluconazole and acyclovir.
Results: Median age was 50 years (range: 36-63). Sixteen patients (80%) had advanced stage II/III MM. Median of previous lines of therapy was 4.5 (range: 1-6). All patients had received at least one prior autologous transplantation. Ten transplants were from a sibling donor and 10 from unrelated donors. Median follow up was 12.5 months (range: 1-37). Median hospital stay was 33 days (range: 26-41). No graft failure was observed. No patient had severe toxic or infectious complications during the admission. Acute GVHD developed in 8 patients (40%) (all grade II), and chronic GVHD in 6 patients (30%). Five patients (25%) required re-hospitalization. After allografting, 7 patients (35%) achieved complete remission, and 5 (25%) achieved partial remission. Transplant related mortality was 10 % (2 patients), one due to septic shock (at day +104), and one due to refractory progressive G-I GVHD (at day +216).
Conclusions: Allo-SCT with fludarabina-melphalan-ATG showed to have a beneficial immunological effect for patients with multiple myeloma (graft versus myeloma activity). In addition, the procedure was associated with a low mortality rate for heavy-treated patients with a high average age, undergoing a second or third transplant.

P896
Peripheral stem cell apheresis in multiple myeloma in the era of new agents
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Objectives: High dose melphalan supported by autologous peripheral stem cell transplantation (APSCT) is the standart treatment in multiple myeloma(MM). The amount of infused CD34+ cells is the major parameter determining the hematopoietic recovery after transplantation. Patient's age, previous treatments and mobilization protocol can affect the success of mobilization. The aim of this study was to investigate the effects of different protocols on the stem cell mobilization.
Methods: Stem cell mobilization procedure was started if the patients had ≥ partial response after ≥ 1 step anti-myeloma therapy. Either granulocyte colony stimulating factor (GCSF) alone or chemotherapy (CT) + GCSF was used. Apheresis was started according to the peripheral CD34 count (>10/µl) on day 4/5 after GCSF alone (GCSF 10 µg/kg/day) or when leucocyte count >1000/mm^3 after a nadir of CT + GCSF. The targetted CD34+ cell count was ≥2x10^6/kg, ideally ≥5x10^6/kg and 8-9x10^6/kg for two autologous transplants.
Results: 142 peripheral stem cell mobilization procedure was performed on 137 MM patients (F/M= 59/78; median age=56 (range 34-72)). 101 patients received GCSF alone and 41 received CT+GCSF. The CT protocols used for mobilization were cyclophosphamide (CY) (n=19), CY+Etoposide (n=7), CY+bortezomib+dexamethasone(n=6), Dexamethosone+CY+Etoposide+Cisplatin (n=6) and Vincristin+Adriamycine+Dexamethosone (n=2) When patients mobilized for the second time after one APSCT and patients mobilized with plerixafor excluded, ≥5x10^6/kg and ≥2x10^6/kg CD34+ cells could be collected in 79.8% and 98.5%, respectively. 4 (3%) patients had mobilization failure; 2 had received APSCT and 1 had received 6 courses of lenalidomid before. The amount of CD34+ cells mobilized after CT+GCSF were significantly more than GCSF alone (median 7.16 (0.65-15.9) vs 8.85

(2.5-58.8) x10^6/kg; p=0.001). There was no significant difference between patients who received bortezomib based protocols before mobilization and who did not in terms of CD34+ cell mobilization (median 7.44 (1.07-58.8) vs 7.27 (0.65-32.9) x 10^6/kg; p=0.803).
Conclusion: Treatment with new agents (e.g. bortezomib) before mobilization or as a part of the mobilization protocol does not seem to have negative effect on stem cell mobilization. Our experience on limited number of patients also demonstrate that a second mobilization is feasible after ASCT. CT+GCSF or GCSF+plerixafor can be used for mobilization failure.

P897
Autologous stem cell transplantation following heart transplantation for AL amyloidosis
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Aim of the study is to evaluate the outcome of three patients with amyloidosis AL who received orthotopic heart transplant (HT) followed by autologous stem cell transplantation (ASCT).
Case 1: 44 years old man diagnosed with cardiac amyloidosis in 2008 underwent HT in July 2009 and ASCT 12 months after. Peripheral Stem cell harvest was obtained with HD-ARA-C and G-CSF. Melphalan (140 mg/mq) was used as preparative regimen in a single administration, followed by infusion of 5.4x10^6/kg CD34+ cells, after DMSO removal. Neutrophil engraftment was reached at day +16; treatment related toxicity was represented by grade II mucositis and transient elevation of creatinine level up to 2 mg/dl. At day +109 patient is in complete remission, with good cardiac and renal function.
Case 2:46 years old woman received a orthotopic HT for AL amyloidosis with cardiac impairment three months after diagnosis; stem cell were harvested with G-CSF stimulation and ASCT was performed 5 months after HT, using Melphalan (140 mg/mq); 5x10^6 CD34+ cells were infused after 48 hours. Early complications were CMV reactivation (day +11) and periengraftment respiratory distress syndrome (day +14). Neutrophil engraftment was observed at day +15. At day +100 free lambda light chains dropped from 776 mg/dl to 99.4 mg/dl, serum creatinine stabilised on 1.7 mg/dl, NT-pro-BNP fell from 3032 to 551 ng/l. With a follow up of 3 years cardiac function is good and serum parameters are stable.
Case 3: 46 years old woman was diagnosed with AL amyloidosis with cardiac disfunction and 10-15% bone marrow plasmacells; she underwent 2 cycles of chemotherapy with Melphalan and Dexamethasone and then two cycles of therapy with Bortezomib and Dexamethasone, achieving a good partial response; 16 months after diagnosis she underwent a orthotopic HT. Peripheral stem cell harvest was performed by stimulation with G-CSF; Melphalan at the dosage of 140 mg/m2 was used as conditioning regimen to ASCT, and 4.22x10^6/kg CD34+ cells peripheral stem cells were infused in two consecutive days. Neutrophil engraftment was observed at day + 12 and early toxicities were represented by moderate hepatotoxicity and grade I mucositis. At one year from transplant serum immunofixation is negative, serum creatinine and NT-pro-BNP are normal.
Our experience in this subset of patients is encouraging; moreover our data show that peripheral stem cell harvest can be safely performed using high dose chemotherapy and G-CSF.

P898
Double early or late salvage auto stem cell transplantation in multiple myeloma patients
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Only few publications address efficacy and safety of double auto stem cells transplantation (ASCT) in multiple myeloma patients. We made a retrospective analysis of 51 patients with double ASCT performed in our centre between 2000-2010. We have analysed patients with planned, second ASCT performed up to 6 months after first ASCT (tandem) and late ASCT applied as a salvage therapy in relapsed multiple myeloma patients. The median time tandem transplant was 5 months (range 3-6) and between double as a salvage therapy was 20 months (range 13-74). Tandem ASCT was performed in 32 patients and salvage ASCT in 14 pts, 5 pts received 3rd ASCT. Median age at the time of tandem ASCT was 50.5 years (range 34-65) and salvage ASCT 52.0 years (range 43-58). Median number of prior lines of therapy was 3 (range 2-4) for tandem group and 6 (range 3-8) for salvage group. Majority of patients in tandem group has been treated with VAD (vincristine, adriamycin, dexamethasone) or thalidomide + dexamethasone. The patients from salvage group received CTD (cyclophosphamide, thalidomide, dexamethasone) or PAD protocol (bortezomib, adriamycin, dexamethasone). All patients from tandem group used stored peripheral stem cells, in salvage group 20% of patients were remobilized using etoposide regimen. Conditioning regimen used for both group was melphalan 200 mg/m^2. Characteristics of response and toxicity in tandem and salvage ASCT in tab. 1 and 2 are presented. Double ASCT is safe and effective procedure which should be reconsidered in multiple myeloma relapsing patients especially in pts in whom relapse occurred after 1 year from first ASCT and may be performed even more than two times. Double ASCT is effective with acceptable toxicity both in early phase of disease and as a tandem procedure and in late phase as a salvage therapy.

Table 1. Tandem and salvage ASCT clinical characteristics of response before second transplant and after 100 days.

Characteristics of response	Tandem ASCT		Salvage ASCT	
	before	after 100 days	before	after 100 days
CR %	20.8	66.7	9.4	35.7
VGPR %	12.5	6.7	14.3	16.7
PR %	45.8	16.7	28.6	33.3
EFS months	54.39		10.98	
OS months	74.12		63.26	

Table 2. Toxicity of tandem and salvage ASCT

Toxicity of procedure	Tandem ASCT	Salvage ASCT
TRM %	3.8	14.3
Sepsis %	3.3	7.2
Mucositis 3-4 WHO %	33.3	28.6

P899
Induction of OPN by HGF in multiple myeloma cells and induction of MMP9 expression by OPN via PI3K/AKT pathways in BMSCs
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Various factors present in the bone marrow microenvironment affect the progression of multiple myeloma. Hepatocyte growth factor (HGF), one of these factors, as a c-Met ligand, activates mitogenic activation protein kinase (MAPK). OPN binds with integrins and CD44 variants and activates cell signaling involved in invasiveness, angiogenesis and bone remodeling. Increasing lines of evidence provide that HGF and OPN, respectively, play a critical role in the progression of multiple myeloma (MM). However, mechanisms by which HGF

modulates the expression of OPN associated with induction of matrix metalloproteinases still remain unknown. In this study, we found that HGF released from bone marrow stroma of multiple myeloma patients was correlated with the expression levels of OPN in multiple myeloma cells. HGF activated MAPK and PI3K/AKT pathways but the expression of OPN mediated by HGF was not suppressed by PI3K inhibitor (LY294002) but by MEK inhibitor (PD98059). Besides, we observed that MMP-9 mRNA was increased in bone marrow stromal cells (BMSCs) treated with OPN. However its expression was suppressed by PI3K inhibitor (LY294002). Our data show that PI3K inhibitor effectively inhibited the MMP9 mRNA expression in both OPN alone treatment and co-treatment with OPN and HGF in BMSCs. Induction of OPN by HGF in MM cells could be one of the major factors for the progression of MM through interaction of MM cells and BMSCs.

P900

Non-myeloablative allogenic haematopoietic stem cell transplantation is a possibly curative strategy for patients with multiple myeloma in remission

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State of the art: The role of allogenic haematopoietic stem cell transplantation (AHSCT) in multiple myeloma (MM) is uncertain. In historical reports, a high transplant-related mortality and a low survival have been described after AHSCT with ablative conditioning regimens.
Study design: We retrospectively reviewed the outcome of 25 patients with MM receiving an AHSCT in our hospital between 2001 and 2009. Results were analysed with SPSS statistical package.
Patients and characteristics: Median age was 55 years (range: 35-64) and 15 patients were male. Patients received a median of 2 therapy lines prior to AHSCT and 19 underwent autologous HSCT previously. Median time between diagnosis and AHSCT was 35 months. Six patients were in complete response (CR), 7 in partial response (PR) and 12 were refractory (REF) at AHSCT time. All patients underwent non-myeloablative conditioning regimen (20 FluMel, 3 Bu2Flu). Donor was HLA identical in all cases (4 non-related). Fourteen patients received bone marrow-HSC and 11 peripheral blood-HSC. As graft-versus-host disease (GVHD) prophylaxis, cyclosporine combined with methotrexate (14 patients) or with mycophenolate (11 patients) was administered.
Results: Median follow up was 15 months (range: 1-86). Overall survival (OS) at 60 months was 35%; those who reached CR at AHSCT time showed a significantly higher survival (p=0.014) compared with those who were in PR or REF (80%, 68% and 10% respectively at 60 months). We observed a higher OS in patients treated before AHSCT with new agents (bortezomib or IMIDs) than in patients exclusively treated with chemotherapy (75% versus 35%, p=0.14). Twelve patients (48%) achieved CR after AHSCT. Acute and chronic GVHD incidence was 32% and 36% respectively. Acute GVHD development was associated with a lower 2-year OS (78% versus 65%, p=0.064). Transplant related mortality (<100 days) was 20% (GVHD± infection) and all of these deaths occur in patients with REF disease. At present, 11 patients (48%) are alive (6 in CR, 4 in PR and 1 REF) and 13 died [MM: 6 patients (46%), GVHD±infection: 3 patients (23%) and other causes: 4 patients (31%)]. Ten of thirteen deaths occurred in patients that were REF.
Conclusions: We conclude that AHSCT can be considered a curative strategy with an acceptable mortality rate in patients with MM who achieve good response (CR or PR) prior to AHSCT. However, this procedure does not improve neither survival nor response rates in REF patients.

P901

High-dose therapy with autologous transplant support in 140 patients with multiple myeloma over 11 years (1998-2009)

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Introduction: high dose therapy (HDT) with aulologous hematopoietic stem cell (HSC) is still effective treatment of MM in young pts under 65 years of age. On 11 years period (April 1998 to December 2009) 140 pts with MM underwent HDT with autologous HSC that represent 38% of all autologous transplantation (140/373) in our center during this period.
Material and methods: this retrospective study include 140 pts: 67 males and 73 females (sex-ratio 0.92); median age 52 years (24-65); Durie and Salmon stage III is observed in 120 pts (86%) with stage IIIB in 17 pts (15%); one pt with plasma cell leukemia; the distribution of monoclonal protein type is IgG in 87 pts (62%), IgA 27 pts (20%), light chain 17 pts (12%), no secretion 6 pts (5%), IgD one pt and no determined 2 pts. Initial standard chemotherapy include 4 to 6 cycles of VAD in 135 pts and association Thalidomide and Dexamethazone in 5 pts. Complete response (CR) was observed in 48 pts (35%), partial response in 57 pts (41%) and refractory disease in 35 pts (25%). Two HDT regimen was used: Mel 200 protocol (Melphalan 200 mg/m^2) for 89 pts (64%) and BU-Mel protocol (Busulfan 12 mg/Kg and Melphalan 140 mg/m^2) for 41 pts (36%). Peripheral HSC was collected by cytapheresis after mobilization by G-CSF alone; median CD34+cells infused was 4,22.10^6/Kg (0,63-19,6). Median follow up is 40 months (6-140).
Results: Early deaths occurred in 6 pts (5%) by TRM. Within 134 appraisable pts, 102 (76%) observed CR, 28 pts (21%) a good response and 4 pts (3%) still on refractory disease after graft. HDT with autologous improve CR rate from 35% to 65% after graft. Also, refractory disease rate decrease from 25% to 3% after graft. Progression disease observed in 81 pts (60%) during follow up with maximal at first 24 months after graft and the same frequency is noted with the two protocol regimen of HDT (Mel200 and Bu-Mel; 60% and 61% respectively). 74 pts (56%) are alive: 44 pts (33%) with persistent response and 30 pts (23%) with relapse; 60 pts (45%) died of progression disease for 58 pts and other reason for 2 pts. The overall survival is 25% at 11 years with a median survival at 42 months.
Conclusion: our results are inferior to Fermand (1999) and Morgan (2000) studies whom treat with VAD and found median survival at 55 months. The most of pts in advanced stage (85%) in our cohort can explain this difference. Actually, initial standard therapy in first intention other than VAD is necessary to improve our results.

P902

Hypoferritinemia prior to autologous stem cell transplantation: the role of underlying malignancy

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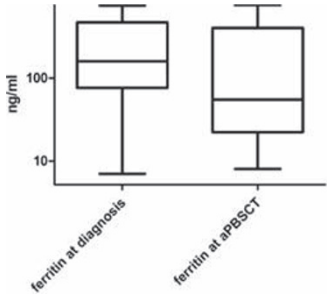
Patients undergoing autologous peripheral blood stem cell transplant (aPBSCT) at our Institution received a work up in order to evaluate eligibility for transplant. Laboratory parameters include iron assessment including iron level and ferritin. The recent occurrence of unusual low serum ferritin concentration in multiple myeloma (MM) patients induced us to study this phenomenon. We collected data on iron assessment in patients undergoing aPBSCT affected by MM or lymphoma (L) from June 2006 to May 2010. We now report 83 patients (46 MM,

37 L), 34 females and 49 males with a median age of 58 years (range 41-65). The comparison was performed using T-test for continuous variables and chi square for categorical factors; p value 0,05 was considered significant. According to laboratory normal range (24-336 ng/ml) we considered as hypoferritinemic patients showing serum ferritin concentration lower than 24 ng/ml. We found 13 patients (out of 83), 15.5% showing low ferritin level. There were 11 patients affected by MM and 2 by L, 11 females and 2 males with a median age of 59 years (range 27-65) showing median serum level of 13 ng/ml (range 3-22). This observation was not caused by differences in terms of age and comorbidities. We observed a significant predominance of female sex in hypoferritinemic patient group even if all female patients were in menopause. Interestingly, comparing MM patients with L patients we observed a significant difference between the two groups in terms of hypoferritinemia incidence (23.9% vs 5.4%, p=0.0211). When ferritin level was available at diagnosis and at aPBSCT a significant reduction was observed (p=0.0452), Figure 1. Usually iron deficiency is caused by gastrointestinal (GI) bleeding, prolonged menstrual cycle or poor oral intake. In our series GI endoscopy was carried out in case of iron depletion and no concurrent disease was detected. No patients received erythropoietin at diagnosis or later during treatment. Thus the reason for hypoferritinemia is currently unknown. We hypothesized that the underlying disease and prior therapy were responsible for the hypoferritinemia, and particularly the cytokine network involved in the pathogenesis of anemia in the MM patients through molecules like hepcidine. Effective MM therapy could interrupt this process leading to massive iron utilization. The role of new drugs (bortezomib, IMiDs) is currently being investigated focusing on hepcidin, IL6 etc role in this phenomenon.



P903

Reduced-intensity allogeneic haematopoietic stem cell transplantation for multiple myeloma

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Introduction: Although allogeneic hematopoietic stem cell transplantation (HSCT) is considered to have the potential to cure multiple myeloma by harnessing the graft-versus-myeloma (GVM) effect, the high transplant-related mortality (TRM) rate offsets its benefit. TRM can be reduced to 20-30% by employing reduced-intensity conditioning (RIC). However, further reduction of TRM is required to evaluate the true GVM effects of allogeneic HSCT. In the present study, we evaluated the safety and efficacy of allogeneic HSCT using RIC for patients with heavily treated or refractory multiple myeloma.
Patients and methods: Fifteen patients with a diagnosis of multiple myeloma who underwent allogeneic HSCT at Keio University Hospital were retrospectively evaluated. Their median age was 45 (range: 33-54). All but one patient, who was a poor mobilizer, had a history of prior autologous HSCT (1 HSCT, n=13; 2 HSCT, n=1). Disease status at allogeneic HSCT was near CR or very good PR in 5 patients, PR in 6 patients, and PD in 4 patients. Stem cell sources were bone marrow (BM) or peripheral blood stem cells from HLA-identical sibling (5 patients), BM from serologically HLA-matched unrelated donor (9 patients, including 2 patients with DRB1 allele-mismatched donor), and BM from a serologically HLA-mismatched unrelated donor (1 patient). Conditioning consisted of fludarabine (25 mg/m² x5) and melphalan (70 mg/m² x2). Cyclosporine A (for HLA-identical sibling) or tacrolimus (for other alternative donors) with short-term methotrexate was given for GVHD prophylaxis.
Results: Engraftment and full donor chimerism were achieved in all patients. Acute GVHD of grades greater than II developed in 6 patients (40%) (II in 4, III in 2), and chronic GVHD developed in 6 patients (40%: limited-type, 2; extensive-type 4). Toxicity of conditioning and GVHD were generally well tolerable. No cases of TRM were observed, and causes of death were all disease relapse or progression (n=4). Three-year estimated overall survival and progression-free survival rates were 58.6% and 18.2%, respectively.
Conclusion: Although there was not a single case of TRM, progression-free survival shows a continuous attrition. These results suggest that reduced-intensity allogeneic HSCT has little potential to cure multiple myeloma or improve its progression-free survival. Further studies of post-transplant therapy, including novel agents, DLI, vaccination, and DC therapy, should be considered.

P904

Intensive therapy with bortezomib and melphalan and autologous stem cell transplantation for patients with multiple myeloma

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Objectives: Upfront high-dose myeloablative chemotherapy followed by autologous stem cell transplantation (ASCT) is the standard therapy for patients with multiple myeloma (MM) under the age of 65 years . Bortezomib is the first proteasome inhibitor approved for MM therapy. The efficacy of bortezomib has been shown in several previous clinical trials in recent years. In addition, the efficacy of administration of bortezomib and melphalan has suggested synergistic effects. Therefore, we combined bortezomib and high-dose melphalan as a conditioning regimen for ASCT in patients with MM, in order to observe the efficiency and safety of the study.
Methods: Fifteen patients with MM received ASCT from Dec 2007 and Nov 2010. Eligible patients were 33– 61 years of age with IgG type (n=5), IgA type (n=2), IgD type (n=1), lamda light chain (n=5), kappa light chain (n=2). The conditioning regimen consisted of intravenous infusion of bortezomib 1.0 mg/m^2 on days -6, -3, and +1, +4, and melphalan 140 mg/m^2 (day-2).
Results: Five newly diagnosed patients received 4-6 cycles of vincristine, adriamycin, and dexamethasone (VAD) combination chemotherapy as induction treatment, Other ten patients received 3-6 cycles consisting of bortozomib, dexamethasone. The disease statuses of these 15 patients at the time of ASCT were 4 complete remission (CR), 10 very good partial remission (VGPR), and 1 partial remission (PR). All patients except 1 had hematologic recovery after ASCT. The median time for the absolute neutrophil counts to increase over 500/mm^3 was 15 days (range, 12-19 days). None experienced transplant related mortality. Adverse events included thrombocytopenia, leucopenia, peripheral neuropathy, fatigue and diarrhea. All of these adverse reactions could be controlled with routine supportive treatment. Ten patients with VGPR or PR at the time of transplantation showed a better response to achieve CR after transplant. The patients were followed up after ASCT with maintenance treatment of thalidomaid. The median follow-up duration was 10 months (range, 1.0-48 months), the overall survival was 93%(14/15),and CR was maintained in 10 patients.
Conclusion: Intensive conditioning regimen consisting of bortezomib and melphalan may be effective and safety in ASCT for MM; however, the feasibility of this regimen should be further evaluated in large study populations.

P905

Autologous stem cell transplant in multiple myeloma patients after treatment with thalidomide/dexametasona or vincristine, doxorubicin, dexametasone

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Introduction: Survival of patients with multiple myeloma (MM) has been extended as a result of high dose therapy with melphalan and autologous stem cell transplantation (ASCT). Thalidomide /dexamethasone (TD) is currently accepted as front line treatment, replacing regimens as VAD (Vincristine, Doxorubicin, dexamethasone) which require hospitalization for the administration of the drugs and the treatment of possible adverse events. Objetives: to compare the outcome of 2 different groups of patients (pts) who underwent ASCT after TD or VAD as induction treatment.

Patients and methods: we retrospectively analyzed medical registries of 57 MM pts who underwent ASCT at our institutions from January 2005 to September 2010. Age: median: 54y (range: 26-70y) Sex:M/F:33/24. Forty pts were in complete remission (CR) at ASCT. Induction treatment: TD for 4 cycles in 38 pts, and VAD regimen for 4 cycles in 19 pts. Peripheral Blood Stem Cells(PBSC) were collected after mobilization with cyclophosphamide and G-CSF.

Results: See the Table.

Discussion: In our group of patients we observed no significant difference in terms of 3 years OS and number of CD34+ cells collected in patients receiving first line therapy with TD or VAD. Nevertheless, TD patients, had a faster hematopoietic recovery and a shorter hospitalization period, which reduces costs of these expensive treatments.

Conclusions: 1- In this group of patients, the use of ASCT as part of first line treatment is associated with a 3 years OS of 80 %. 2- The addition of novel agents as part of the first line therapy could improve these results. 3- The use of TD as first line therapy before ASCT compares favourably in terms of costs related to the treatment of these patients.

	Tal/ Dexa	VAD	
CD34+ x 10^6 kg.collected	9.16	10.22	P= 0.5
Neutrophil engraftment > 500/cc	11.97 days	13.3 days	p= 0.034
Platelet engraftment >20000/cc	14.8 days	19.7 days	P= 0.011
Hospitalization days	21.4	24.2	P= 0.013
Follow up	658 days (93-1063)	1208 days (104-3524)	
3 years OS	82%	81 %	P= 0.37

P906

Treatment of multiple myeloma with autologous stem cell transplantation: a long-term observation

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Introduction: High-dose melphalan followed by autologous stem cell transplantation (ASCT) is wildly used as intensification treatment in pts with multiple myeloma (MM) responsive to the initial chemotherapy. However, there is grooving evidence that only the subset of patients who achieve complete remission actually benefit from this approach.

Methods: From June 2000 to June 2010 87 pts with MM (49 male, 38 female), median age 52,2 (range 29-68) were transplanted in the department Bone Marrow Transplantation. There were 50 pts with myeloma G, 15 with myeloma A, 16 with light-chain myeloma, 1 with myeloma D, 1 with myeloma M, 2 with non-secretory myeloma and 2 pts with plasma cell leukaemia. According to Durie-Salmon 58 (67%) pts were in stage III and 29 (33%) pts were in stage II. Pts received VAD chemotherapy and/or bortezomib + dexamethasone ± doxorubicin as induction. Stem cells were mobilized with cyclophosphamide (4-6 g/m^2) and G-CSF. HDT/ASCT entailed conditioning with melphalan 200 mg/m^2. Response was defined according to IMWG criteria.

Results: After the induction the overall response (CR+ VGPR+PR) was 91% with 18% of CR and 38% of VGPR. During mobilization a median of 18,5x10^6 CD34+ cells/kg were collected (2,1-105x10^6). The median of apheresis was 2 (1-6). In all pts mobilization was successful. Double HDT/ASCT was performed in 54 pts and single in 33 pts. In 23 cases the second ASCT was not performed due to complications after the first ASCT or patient's refuse and in other 10 cases the second ASCT is planned. There was no treatment related mortality. Patients were evaluated on day 90 after the end of HDT. 48% of them obtained CR, 29% VGPR and 14% PR and 9% were in SD.

The median time of follow-up after ASCT was 12,5 months (5-103,7). The OS at 7-year was 71%. Median of PFS was 45 months. PFS at 3-year was 55%, at 7-year 19%. 75 pts (86%) are still alive.

The patients in CR/VGPR before ASCT had significantly longer OS and PFS compared with those in PR (p=0,03 and p=0,001). In pts with CR/VGPR OS at 5-year was 78% and PFS at 5-year - 69%. In 10 pts the second HDT/ASCT was performed in progression. In those cases OS at 20 months was 46% and PFS – 31%.

Conclusion: HDT followed by ASCT is a safe well tolerated method of treatment for pts with MM who can obtain long-lasting survival. Our findings suggest that patients who are in CR/ VGPR before ASCT have a better PFS than those in PR before ASCT.

P907

Multiple myeloma – Evolution and survival in patients after ASCT according to the induction therapy. Data of CIC 859, Sofia, Bulgaria

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Introduction: ASCT is a standart of therapy for patients with multiple myeloma under 65 years old. High-dose chemotherapy (HDC) with autologous stem cell transplant (ASCT), has recently been shown to increase rates of overall response, survival, and event-free survival administreated for suitable patients. Prior to HDC patients receive 3-4 cycles of induction therapy. Induction treatment with VAD is curently being replased by new targeted agents (Velcade) with high anti myeloma activity. The novel agents may increase the CR /VGPR rate before ASCT, which may improve post transplantation responce and OS.

Patients and methods 44 patients (female/male ratio -15/29) with MM were analysed, mean age 52.75y. (39-67), some received bortezomib-containing regimens (n=21), others VAD (n=23) before ASCT. All patients received peripheral stem cells, mobilized after G-CSF (n=35), HD Endoxan + G-CSF (n=5) or G-CSG + Mozobil (n=4). The median duration from diagnosis to ASCT was 20 months (range, 5–61 months) for all patients.

Results Overal response rate (at least a partial response) prior to ASCT was documented in 20 (95.2 %) of bortezomib-containing regimens and in 20 (86.5%) of patients with VAD chemotherapy. The difference between the two groups was not significant (P = 0.608). The responce assesment after ASCT for bortezomib containing regimen was; CR in 9 (42.8%), VGPR 9 (42.8%) and PR in 3 (14.2%). The responce assesment for the VAD group was: CR in 3 (13.04%), VGPR in 13 (56.5.1%), PR in 6 (26%), 1 patient died at day +15 (TRM -2.2%).

However, the high-quality response rate with VGPR or more in the bortezomib group was higher compared with the VAD group

(85.7 % vs 69.9%, respectively, (P=0.28). The engraftment data as well as stem cell harvesting were comparable between the two groups. After ASCT, the difference between the two groups did not reach the level of statistical significance with respect to progression-free survival and overall survival because of the short period of follow up.
Conclusions The results of this retrospective comparison of bortezomib-containing regimens with the VAD as induction treatment prior to ASCT for MM suggest that the choice of induction therapy may be improtant to long-term post- transplant outcomes. Future trials of pre- transplant induction therapies should be designed to analyze differences in progression free and overall survival.

P908

10-year experience in the treatment of multiple myeloma with autologous stem cell transplantation

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Background: Multiple myeloma (MM) is a plasma-cell proliferative neoplasm.The second most common hematologic cancer, with 5 years prevalence about 183 000. Incidence is 5,7 /100 000 in EU. 5-years survival is 28%. Treatment with HDT and single autologous transplantation is a category I recommendation of the NCCN. In young patients, the impact of dose intensity has been demonstrated, and single HDT supported with ASCT using a conditioning regimen with Melphalan should be considered a standard of care. Double transplantation can be proposed to patients failing to achieve a VGPR after a first ASCT.
Material and methods: during a 10 years period we have performed 195 stem-cell transplantation in different hematological malignancies. 34 (17,5%) high dose chemotherapy and autologous stem-cells transplantation were performed in 30 patients (4 tandem transplantation) with multiple myeloma. In this trial we retrospectively analyzed the epidemiology characteristic of this patients. Gender: Female: 16 Male: 14. Median age: 51 years (from 43- 64 years).
Results: Diagnose was made according to Salmon and Durie criteria. 25 patients with IgG, 4 with IgA, and 1 with light chain myeloma. Bence-Jones positive myeloma was diagnosed in 8 patient, 5 of them were with chronic renal failure. Fracture of spine was presented in 12 patients and 2 patients has fracture of hip. For the induction of remission we used VAD regimen in 20 patients, Cy-Tal-Dex in 10 patients. As a second line therapy in the case of failure to achieve complet remission we introduce Thal/Dex regimen. in 10 patient Only in two patient we use Bortezomib, Alkeran, Dexamethason. Conditioning regimen consisted Melphalan 200 mg/m^2. In tandem transplantation the dose of second conditioning was 140 mg/m^2.The volume of CD34+ cells was 3,88 x 10^8/Kg.bw. Period from diagnose to transplantation is 12 months. From 30 patients 80% are alive, 6 died (3 renal failure, 2 fatal cerebral bleeding and 1 multiorgan failure). The DFS is 24 months, OS is 48 months and survival after transplantation is 35 months.
Conclusion: novel agents such as thalidomide, bortezomib, or lenalidomide have been introduced to improve high-dose therapy, and promising results have been reported. Conversely, results from myeloablative allogeneic stem cell transplantation remain disappointing due to high TRM, justifying the exploration of strategies such as RIC, which have been shown to be feasible but for which proof of efficacy requires continued study.

P909

Salvage therapy with bortezomib in myeloma patients with relapse or progression after stem cell transplantation

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Background: Bortezomid has been demonstrated to be efficacious end well tolerated in patients (pts) with relapse multiple myeloma (MM).
Patients and methods: We have retrospectively analysed MM patients who relapsed or progressed after stem cell transplantation (SCT) and received Bortezomib as salvage therapy.
Results: Between 1994 and 2010, 22 MM pts with median age of 50 years (range 3-65), in relapse or progression after high dose therapy (10 single autologous SCT, 9 double autologous SCT and 3 autologous and allogenic SCT), were treated with Bortezomib and dexamethasone (14 pts) associated with Thalidomide in 8 pts. Median time between SCT and Bortezomib was 31 months (range 5-123). Patients received a median 7 (2-10) cycles of Bortezomib. The overall response rate was 36% (5 CR+VGPR and 3PR). With median follow up of 10 months (range 2-56), 14 pts (63%) are alive (6 CR+VGPR and 4 PR). Eleven patients experienced at least grade 2 or higher haematological and neuropathy toxicities. The most common grade 3/4 AEs includes thrombocytopenia (22%), neutropenia (5%). The overall incidence of neuropathy was 41%, including 23% grade 3.
Conclusions: This study shows that Bortezomib is well-tolerated, feasible and active therapeutic option in patients with relapsed and progressive MM after SCT.

P910

At-home management of aplastic phase following high-dose chemotherapy and autologous haematopoietic stem cell transplantation for multiple myeloma patients: a pilot study

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After high-dose chemotherapy with autologous hematopoietic stem cell transplantation (HSCT) long hospital stays in the aplastic phase are expensive, lead to increased risk of hospital infections and to increasing pressure on available hospital beds. The aim of this pilot study was to analyze the feasibility of a home care program (HCP) regiment for Multiple Myeloma patients receiving high-dose melphalan, and undergoing autologous HSCT to be at home for the aplastic period, without daily hospital visits. The HCP consisted of patients who were discharged the day after stem cell reinfusion, after which specialized transplant physicians delivered all supportive care including transfusions and parenteral antibiotics at home. Sixteen patients consecutively entering the HPC program from June 2010 until to day and the results of the study will be introduced during the next EBMT congress.

P911

Unrelated cord blood transplant for adult with primary acute myeloid leukaemia. A survey by EUROCORD and the Acute Leukaemia Working party of the EBMT

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Patients (pts) with high-risk acute myeloid leukemia (AML) have few chances of cure without allogeneic HSCT. HSCT can be used in first remission for pts with poor-risk cytogenetics, as rescue for pts refractory to chemotherapy, at first relapse or in second and subsequent remission. UCB is an established stem cell source for HSCT. We retrospectively analyzed 604 adult (>18y) with de novo AML in complete remission (CR)1 (n=229), CR2 or CR3 (n=228) and advanced disease (n=147) who received UCBT as first transplant. Pts were transplanted from 2000-2010 in 131 EBMT centers. Median age was 41y, 18% of the pts had previous autologous transplant. Based on available cytogenetic and molecular markers at diagnosis (n=339) 56% were in intermediate risk and 31% in unfavorable risk group.
Grafts were composed of 1 (sUCBT) (n=361) or 2 (dUCBT) (n=243) CB units, 39% of CB units were identical to recipient or had 1 HLA disparity (antigen level for HLA-A and B allelic level for DRB1) while 61% had 2-3 HLA disparities. At infusion median TNC cell dose was $3.1x10^7$/kg ($2.4x10^7$/kg with sUCBT and $3.7x10^7$/kg with dUCBT) and median CD34+, $1.2x10^5$/kg ($1x10^5$/kg with sUCBT and $1.3x10^5$/kg with dUCBT).
Fifty-one percent of pts received a myeloablative conditioning regimen (MAC) and 49% a reduced intensity regimen (RIC). The most common regimens used were busulphan+fludarabine+thiotepa for MAC and cyclophosphamide+fludarabine+TBI2Gy for RIC. GVHD prophylaxis consisted of CSA±MMF in 58% of pts and CSA±steroids in 32%. Median follow-up is 13 months.
Cumulative incidence (CI) of neutrophil recovery, acute GVHD(II-IV) and 1y TRM was 80±2%, 26±3% and 21±3%, respectively. CI of 2y relapse was 38±3% (27% CR1, 29% CR2 and CR3, 56% advanced, p=0.001). It was 31% for those pts transplanted with MAC (n=291) and 30% with RIC (n=282). The 2y probability of leukemia-free-survival (LFS) was 33±2% (45% CR1, 41% CR2, 16% advanced, p <0.001). In pts given a MAC, 2y LFS was 50% for CR1, 27% for CR2 or CR3 and 17% for more advanced phase of the disease whereas it was 35%, 44% and 18% for RIC respectively.
Causes of death were infections or other transplant-related events (n=195) or disease progression (n=149).
In conclusion this large series of pts show that UCBT is an option treatment for adults with high risk AML after a myeloablative or reduced conditioning regimen without an HLA identical donor. Data on cytogenetics and molecular markers are being collected in order to perform a risk factor analysis.

P912

Prognostic factors and outcomes of adults with acute lymphoblastic leukaemia who relapse after allogeneic haematopoietic cell transplantation. An analysis by the Acute Leukaemia Working Party of the EBMT

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Small case series indicate that adults with ALL who experience relapse after allo-HCT have a dismal outcome. However, data on treatment strategies after relapse and their outcomes are missing. We performed a retrospective analysis of patients with relapsed ALL after allo-HCT transplanted in CR and the strategies adopted by EBMT centers. 84 centers completed the specific questionnaire of 465 adult patients with ALL (76% B-ALL, 21% T-ALL) who relapsed after a first allograft (BMT 47%/ PB 53%, myeloablative 92%/ RIC 8%) performed in remission (CR1 65%, CR2/3 32%/3%). Median time from transplantation to relapse was 6.9 months (0.8-57). Salvage treatments were:
1) supportive care in 13% pts,
2) cytoreductive therapy including chemotherapy and/ or radiation and/or tyrosine kinase inhibitor (61% of the Ph+ cases) in 43%:
3) DLI and/or cytokines in 23% pts, and
4) second allogeneic HCT in 20% pts.

With a median follow up of 46 months (1-117), the median survival after relapse was 5.5 months. The estimated 1-, 2- and 5-year post-relapse survival was 30±2%, 16±2% and 8±1%, respectively. At univariate analysis, age at transplant, disease characteristics [immunophenotype, high risk Ph+ or t(4;11) ALL, donor type, stem cell source and conditioning (intensity, type)] had no significant impact on post-relapse survival. In a multivariate analysis, adverse factors for survival found to be transplantation at advanced CR (p<0.012, HR:0.74, 95% CI: 0.59-0.94), early relapse (<6.9 mo.) after transplant (p<0.0001, HR:1.66, 95% CI: 1.32-2.10) and peripheral blast percent at relapse (>10%) (p<0.0001, HR:0.56, 95% CI: 0.44-0.70). Inclusions of post-relapse therapies as time-dependent variables in the Cox analyses showed that DLI or second transplantation were not significant prognostic indicators for survival after relapse. Based on the multivariate model for survival after relapse we 3 groups of patients were identified if they have 3, 2 or 0/1 of the above adverse factors. In fact 2 year survival was 6±2%, 17±3% and 30±7%, respectively. In conclusion, our study emphasizes the grim prognosis for patients with relapsed ALL after allo-HCT and highlights the need for new therapies. The prognosis of relapsed patients seems to be determined more by relapse characteristics rather than by features at diagnosis. We identified a subgroup of patients with none or 1 adverse factor which are most likely to benefit from post-relapse therapy.

P913

Reduced-intensity conditioning unrelated donor allogeneic stem cell transplantation for acute lymphoblastic leukaemia: a retrospective survey from the European Group for Blood and Marrow Transplantation

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In older patients with acute lymphoblastic leukaemia (ALL) allogeneic stem cell transplantation (allo-SCT) offers long term disease control, however these patients may be ineligible for myeloablative transplantation due to age or co-morbidity. Recently, reduced intensity conditioned (RIC) sibling allo-SCT has been shown to offer similar leukaemia-free survival (LFS) to myeloablative conditioning. As most patients will not have a suitable sibling donor we have examined the outcome of

RIC allo-SCT from matched unrelated donors (MUD) in ALL. 241 patients aged 18 years and above received a minimum 6/6 human leukocyte antigen (HLA) matched MUD RIC allo-SCT between 2000 and 2009, median year of transplant was 2007. Median age was 52 (range 18-72) years, 55% were male. The majority (n=197, 82%) were in complete remission (CR) at transplant (CR1 56%, CR2+ 26%) the remainder had relapsed or refractory disease (RRD). Data on BCR-ABL rearrangement were available for 172 patients, BCR-ABL was present in 74 (43%) of these. Reasons for RIC as available for 122 transplants were: recipient age (n=41), local protocol requirement (n=38), co-morbidity (n=29), prior SCT (n=6), infection (n=2) and other (n=6). Following SCT 224 patients (94.5%) engrafted. Incidence of grade II-IV acute GVHD was 29%. With a median follow-up of 13 (range 1-112) months estimates of 2y overall survival (OS) and LFS were 35±4% and 31±3%, respectively. Relapse incidence (RI) and non-relapse mortality (NRM) at 2y were 37±3% and 32±3%, respectively. In univariate analysis more advanced disease status at transplant was associated with impaired LFS (39±5%, 28±7% and 7±5% for CR1, CR2 and RRD, p<0.0001) and higher RI (30±5%, 47±7% and 49±8% for CR1, CR2 and RRD, p=0.005). NRM was also higher for patients with RRD (31±5%, 25±5% and 44±8% for CR1, CR2+ and RRD, p=0.051). Age above median was not associated with impaired survival, increased RI or NRM. Patients transplanted after 2007 were significantly older (median 55 vs. 48 years, p=0.03) and fewer had RRD (14 vs. 23%, p=0.07). After 2007 LFS was not significantly different (32±6% vs. 29±5% p=0.41) but RI was significantly lower (27±5% vs. 46±5%, p=0.01), this was balanced by an increase in NRM (41±5% vs. 25±4%, p=0.06). The presence of BCR-ABL did not influence LFS or RI. Early RIC MUD allo-SCT for patients in CR is effective in both BCR-ABL positive and negative ALL.

P914

Intermediate dose of anti-human thymocyte immunoglobulin reduces transplant related mortality with no impact on the relapse rate of acute leukaemia patients undergoing allogeneic transplantation after a myeloablative conditioning regimen

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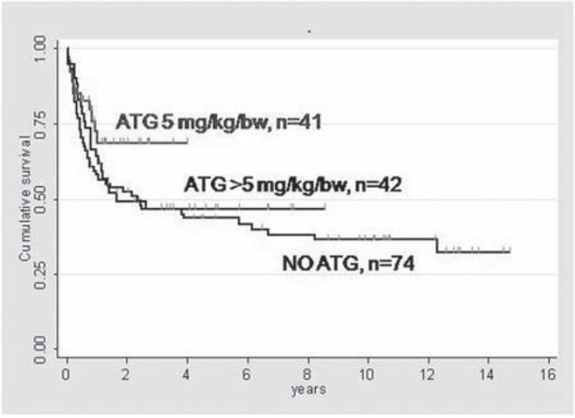
Background: The addition of anti human thymocyte immunoglobulin (ATG) to GVHD prophylaxis results in decreased incidence of acute and chronic GVHD after allogeneic haematopoietic stem cell transplantation (alloHSCT). However, its use may delay T-cell reconstitution, prolong susceptibility to opportunistic infections and increase leukaemia relapse. The main purpose of this study was to evaluate retrospectively the impact of different doses of ATG on overall survival (OS), cumulative incidence of relapse (CIR) and transplant related mortality (TRM) of acute leukaemia patients undergoing alloHSCT from both sibling and unrelated donors following a myeloablative conditioning regimen.
Patients and methods: We analysed 157 consecutive patients (median age 37, 85 males) with acute lymphoblastic (ALL, n= 62) or myeloblastic (AML, n= 95) leukaemia who underwent alloHSCT between May 1994 and August 2010. For GVHD prophylaxis, a conventional Cyclosporin A and Methotrexate program (with no ATG) was given to the first 74 patients. ATG (Genzyme, Italy) was given at >5 mg/kg/bw to a second cohort of 42 patients and at the dose of 5 mg/kg/bw to the last 41. The donor was a related sibling in 79 or unrelated in 78 patients. The disease status at transplant was complete remission (CR) in the majority of cases (CR1= 82, CR2= 28) and active disease in 47 cases. All patients underwent myeloablative conditioning (71% TBI based). The stem cell source was bone marrow in 38 patients and peripheral blood in 119 patients.
Results: With a median follow-up of 15.8 months (0.4-178), the 4 years OS, CIR and TRM of the whole patient cohort (n=157) is 48%, 36%, 23%, respectively. As expected, the use of ATG is associated with a reduced incidence of acute and chronic GVHD, no matter the dose used. More interestingly, the 4-years OS of patients receiving ATG at a dose of 5 mg/kg/bw is 69% compared to 47% and 44% for patients receiving ATG >5 mg/kg/bw or no ATG (Figure 1). In this analysis, the use of ATG does not increase the risk of leukaemia relapse irrespectively of the leukaemia phenotype (ALL vs AML) and the donor type (related vs unrelated). A comparable higher risk of TRM is seen in patients who either received no ATG or ATG at more than 5 mg/kg/bw.
Conclusions: For acute leukaemia patients an appropriate dose of ATG may reduce TRM with no detrimental effect on the relapse rate and with an overall benefit on survival.

Table 1

	N	CIR (%)	p	TRM (%)	p	OS (%)	p
ATG							
None	74	35	-	28	-	44	-
5 mg/kg/bw	41	37	0.8	12	0.3	69	0.07
> 5 mg/kg/bw	42	36	0.8	20	0.3	47	0.5

Figure 1



P915

Allogeneic stem cell transplantation for adult Philadelphia chromosome-negative acute lymphoblastic leukaemia in first complete remission: comparisons between related and unrelated, and myeloablative and reduced-intensity conditioning transplantation

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The indication of allogeneic stem cell transplantation (allo-SCT) for Philadelphia chromosome-negative acute lymphoblastic leukemia (Ph(-) ALL) in first complete remission (CR1) is still controversial. Recently, a large prospective donor versus no-donor analysis reported that related allo-SCT for Ph(-) ALL in CR1 could provide the most potent survival benefits. However,

the indication of unrelated allo-SCT for adult Ph(-) ALL in CR1 remain unclear. In addition, there are only a few small studies concerning reduced-intensity conditioning (RIC) allo-SCT for Ph(-) ALL.
We retrospectively analyzed 681 adult Ph(-) ALL patients transplanted in CR1 (335 related and 346 unrelated) using the data provided by the Japan Society for Hematopoietic Cell Transplantation and the Japan Marrow Donor Program. To analyze the efficacy of RIC allo-SCT for Ph(-) ALL in CR1, we compared the outcomes of 21 RIC with 81 myeloablative conditioning (MAC) HLA-matched allo-SCT for patients aged ≥45 performed between 1998 and 2007.
There was no significant difference in overall survival (OS) between related and unrelated allo-SCTs (related vs unrelated: 63% vs 62% at 4 years, respectively; P=.30). In related allo-SCT, relapse was significantly higher (related vs unrelated: 32% vs 22% at 4 years, P=.02), and less than 6 months from diagnosis to allo-SCT and tacrolimus-based graft-versus-host disease prophylaxis were associated with relapse. On the other hand, in unrelated allo-SCT, non-relapse mortality (NRM) was significantly higher (related vs unrelated: 15% vs 27% at 4 years, P=.0002), and 10 months or longer from diagnosis to allo-SCT, HLA-mismatch, and abnormal karyotype were associated with NRM. In 102 CR1 patients aged ≥45, the results were 65% for OS, 62% for leukemia-free survival (LFS), 15% for relapse, and 35% for NRM in MAC, versus 71% for OS, 71% for LFS, 21% for relapse, and 29% for NRM in RIC at 2 years.
Comparable survival rates were observed between related and unrelated allo-SCTs for adult Ph(-) ALL in CR1, although relapse rates, incidences of NRM, and risk factors were different between them. Unrelated allo-SCT could also be a treatment of choice for adult Ph(-) ALL in CR1. Similarly, comparable outcomes were observed between MAC and RIC allo-SCTs. RIC allo-SCT could be a potential therapeutic option for adult Ph(-) ALL patients aged ≥45 in CR1 and not eligible for MAC allo-SCT.

P916
Copy number changes in AML patients undergoing unrelated haematopoietic cell transplantation results in loss of mismatched HLA at relapse
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A combination of continuous immunologic pressure mediated by donor T cells and clonal evolution of the disease can result in acquired genomic aberrations (GAs) after allogeneic hematopoietic cell transplantation (HCT). Acquired GAs can affect chromosomal regions with possible relevance in the transplantation outcome. We investigated genomic aberrations in 21 patients undergoing related and unrelated, HLA-matched HCT in leukemic blasts before transplant and at relapse after transplantation. Copy number analysis revealed a total of 145 genomic abnormalities in 21 pairs of samples before allogeneic transplantation and at relapse in patients diagnosed with de novo AML or secondary AML. Total number of GAs was significantly higher after transplantation compared to the pre-transplant samples ($p<0.05$). Eight out of twenty-one patients showed one or more genomic alterations before transplantation, while in 16 out of 21 patients they were detected only in the relapse sample. Within the group of patients without chromosomal aberrations, only in one patient a small deletion on chromosome 14q11.2 (0.5-0.6 Mb) was observed in the pre-transplant sample which was not detected by conventional cytogenetics. In contrast, in the group of patients with abnormal/complex karyotype, six additional losses and one gain were found in 7 patients. Taking these results together, 17% of GAs were revealed only by SNP array, in 8 (38%) cases. Shared abnormalities, found before transplantation and at relapse, were identified in eight patients (38%) indicating common clonality. Conversely, 23 novel losses, 6 LOH regions and 6 gains arose at relapse in 13 patients (62%).
A very interesting finding in our study was a large homozygous region (40 Mb) spanning the HLA-locus on chromosome 6p which was identified at relapse in two patients. Sequence-based HLA typing of the blasts from these two patients revealed a loss of the patient specific allele at the mismatched locus leading to homozygosity for the HLA haplotype shared by the patient and the donor. Large and cryptic aberrations, detected with array-based copy number profiling, in patients undergoing allogeneic HCT can be taken into account for refining risk assessment and eventually for choosing an appropriate or more efficient treatment for relapse after transplantation.

P917
Myeloablative conditioning with fludarabine and iv-busulfan for allogeneic stem cell transplantation: a comparison with BU-CY2 in acute myeloid leukaemia
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Background: The regimen based on Busulfan (Bu) and Cyclophosphamide (Bu-Cy2) is regarded as the standard myeloablative regimen for allogeneic stem cell transplantation (SCT). Fludarabine (FLU) has been used recently in conditioning regimen associated to myeloablative dose of BF. This study evaluate the role of this myeloablative BF conditioning regimen compared to a series using Bu-Cy2 standard in allogeneic SCT in adult AML.
Materiel and methods: From February 2008 to June 2010, 79 AML pts with a median age of 44 y(18-61). AML pts in first CR (70), in second RC (6) and blastic (3) received an allogeneic SCT with myeloablative BF based on the use of FLU at 200 mg/m² and once daily IV-Bu at 3,2 mg/kg/d (4 d). GVHD prophylaxis associated Ciclosporin (CsA) and Methotrexate. For comparison, a review from September 1999 to April 2010 was conducted in the medical records of 160 pts who received Bu-Cy2 (n=20 pts) and Bu-Cy2-VP16 (140 pts), with a median age of 27 years (18-47); AML pts in first CR (146), in second CR (11) and blastic (3). The same GVHD prophylaxis was used as BF. All the pts received peripheral SCT from an HLA identical sibling donor.
Results: Median time of aplasia was superior in the standard BU-Cy2 group: 13 days (7-26) than in the BF group: 10 days (4-20) (p: 10-6). Acute GVHD was the same in both, with respectively 24,3%(19% grade II-IV) in BU-Cy2 group and 30,6% (25% grade II-IV) in the BF group (p:0,3). Chronic GVHD was also the same with 46,8% (extensive: 26%) in BU-Cy2, versus 53,7% (extensive: 41%) in BF group (p:0,3). The CMV infection was also found in the same rate in both 21% versus 17% (p:0,4). Relapse occurred in 17 pts (11,8%) in Bu-Cy2 and 14 pts (18,6%) with BF (p:0,16). No case of veno-occlusive disease reported versus 9,7% with BU-Cy2 (p:10-6). TRM incidence was 31,2% in Bu-Cy2 group versus 17,7% in BF group (p:0,02). At November 2010, 53 pts (67%) are alive in the BF group and 94 pts (58,7%) in Bu-Cy2 group, with a median follow up of respectively 13 months (4-44) and 78 months (7-128). The OS is 65,5% for BF versus 57,9% for BU-Cy2 group (p: 0,9), the EFS is respectively 59,1% in BF group and 57,5% for BU-Cy2 group (p: 0,9) without significant difference.
Conclusion: According to our results, myeloablative BF conditioning regimen may substitute classical Bu-Cy2 conditioning in AML, especially since it has the advantage of being less toxic, with significant reduction of neutropenia and duration of hospitalization.

P918

KIR2DL2/3 - HLA-C-Group1 and KIR2DL1 - HLA-C-Group2 mismatches improve the clinical outcome in patients with acute myelogenous leukaemia after allogeneic stem cell transplantation

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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 1998 and 2007.
Patients and methods: In a consecutive single center cohort of 147 AML patients, samples from 78 donor/patients, pairs were evaluable for retrospective KIR-ligand matching. The median patient age was 48 (25-67) years. Patients were transplanted with G-CSF-stimulated PBSC (n=74) or bone marrow (n=4) from HLA matched unrelated (n=34) donors or family related donors (n=44). 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: A lack of HLA ligands for donor inhibitory KIR2DL receptors in the recipient (mismatch) was associated with a significantly superior event-free survival (0.58 vs 0.31, p=0.017), a superior overall survival (0.62 vs 0.34, p=0.035), a reduced non-relapse mortality (0.07 vs 0.34, p=0.007), and a reduced incidence for relapse, which did not reach significance. Patients with the mismatch revealed a reduced incidence of grade 3 to 4 aGvHD (p=0.031).
Conclusions: In conclusion, the mismatch for KIR2DL2/3 - HLA-C-Group-1 or KIR2DL1 - HLA-C-Group-2 ligands is associated with a significantly better outcome for AML patients after allogeneic stem cell transplantation.

P919

Central nervous system involvement in adult acute lymphoblastic leukaemia at diagnosis treated with autologous and allogeneic transplantation: a survey from the Société Française de Greffe de Moelle et de Thérapie Cellulaire

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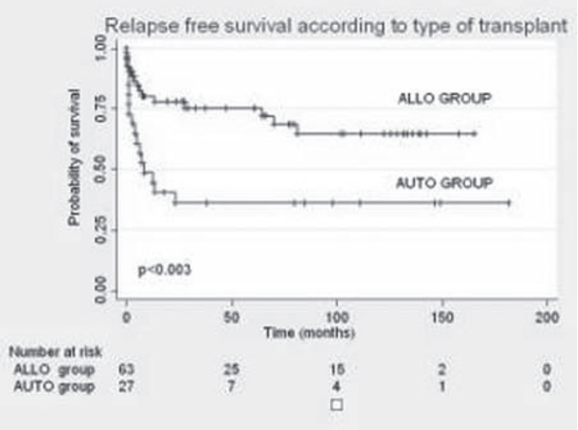
CNS leukemia in adult ALL is uncommon at diagnosis and outcome after transplantation remains unclear.
This retrospective analysis aimed to assess the role of autologous stem cell transplantation (ASCT) or allogeneic stem cell transplantation (alloHSCT) in 90 ALL adult patients with CNS involvement at diagnosis, reported to the SFGM-TC registry between 1994 and 2008. In this series, 27 patients underwent ASCT, while 63 received alloHSCT.
Results : The median follow-up was 22.7 months [range, 0.4-182]. The median age at presentation was 26.2 [range, 15-63] years. ALL immunological subtype were as follow: 39 B-ALL cases, 46 T-ALL and 5 unclassified. At diagnosis, 7 patients (8%) and 10 patients (11%) presented with t(9;22) and Bcr-Abl positivity, respectively. At time of transplantation, 67 patients (74%) were in first CR : AlloSCT 44/63(70%), ASCT 23/27 (85%), 15 (17%) in CR≥2, and 8 (9%) treated with progressive disease. The median time to transplant was 5.7 [range, 2.7-73.1] months. Twenty two patients received cerebral irradiation before transplant (12 in the allo group vs 10 the auto group, p<0.05). Two patients who relapsed after ASCT were secondary allografted, and 3 patients received a second alloHSCT.
Among the 63 alloHSCT patients, 55 patients (87%) received a myeloablative conditioning regimen, including 51 (81%) with high-dose TBI. In the ASCT group, high-dose TBI was used in 26 patients (96%). Thirty eight (60%) patients received alloHSCT from a related donor and 24 (38%) from unrelated donors. Grade 3-4 acute GVHD occurred in 13 cases (21%). Limited and extensive chronic GVHD were reported in 12 (19%) and 10 patients (16%), respectively. The TRM incidence at 60 months was 34% for the AlloHSCT group and 18.2% for the ASCT group (p=ns). The median relapse-free survival was 7.6 and 26.3 months for the ASCT and alloHSCT groups respectively (p<0.003, Fig. 1). However, the median overall survival was similar between the two groups (17.3 vs 26.3 months). Age over 50 y. and absence of acute GVHD were associated with a lower median relapse free survival (p<0.03 and p<0.02, respectively).
In all, we conclude that despite the use of cerebral irradiation, the use of TBI in the conditioning regimen and a lower rate of TRM in ASCT, RFS was significantly higher in the alloHSCT group as compared to ASCT, suggesting a potential role for an immune mediated allogeneic graft-vs-tumor effect in ALL patients with CNS involvement.



P920

Intensified conditioning with total-body irradiation, etoposide, and cyclophosphamide for children with acute lymphoblastic leukaemia in first and second complete remission

H. Yabe on behalf of the Stem Cell Transplantation Committee of Tokyo Children's Cancer Study Group (TCCSG)

Objectives: We used high-dose etoposide (VP-16) in addition to a conventional preparative regimen comprising total body irradiation (TBI) and cyclophosphamide (CY) to improve event-free survival (EFS) after stem cell transplantation (SCT) for childhood acute lymphoblastic leukemia (ALL) in first or second remission.
Methods: One hundred and eight children with ALL were treated with allogeneic SCT using a preparatory regimen comprising 12 Gy of TBI, 60 mg/kg (body weight less than 30 kg) or 1800 mg/m^2 (body weight more than 30 kg) of VP-16, and 120 mg/kg of CY between July 1995 and August 2005. The method for graft-versus-host disease (GVHD) prophylaxis was selected according to the stem cell source: short-term methotrexate (MTX) and cyclosporine (CyA) or CyA alone was used in HLA-identical

related SCT, and both MTX and tacrolimus were administered in alternative-donor SCT. Seventy-one patients underwent SCT in the first complete remission (1CR), whereas 37 patients underwent SCT in the second complete remission (2CR). Stem cells were obtained from an HLA-identical sibling in 32 cases, from a related donor other than an HLA-identical sibling in 10 cases, from an unrelated BM donor in 48 cases, and from an unrelated cord blood in 18 cases.
Results: All patients achieved engraftment, at a median time of 17 days (range 11-44) and 29 days (range 17-177) for neutrophil and platelet recovery, respectively. According to the disease status, the EFS in 1CR was 74% [95% confidence interval (CI): 64–85] and that in 2CR was 65% (95% CI: 49–80). According to the stem cell source, the EFS in 1CR was 71% (95% CI: 53–89) in cases of HLA-identical sibling bone marrow transplantation (BMT) (n = 24), 80% (95% CI: 45–100) in cases in which stem cells were obtained from a related donor other than an HLA-identical sibling (n = 5), 71% (95% CI: 54–88) in cases of unrelated-donor BMT (n = 28), and 85.7% (95% CI: 67–100) in cases of UCBT (n = 14). The transplant-related mortality (TRM) rates according to disease status were 7.5% in 1CR and 8.7% in 2CR. Eighty three of the 108 patients (77%) survived and achieved CR, with a median observation period of 4.6 years (range, 0.9–11.5 years). Early death within 100 days after SCT was observed in 1 patient.
Conclusions: The addition of high-dose VP-16 to TBI and CY regimen can lead to improvement in EFS without increase in the TRM rate even in alternative donor SCT.

P921
Allograft after paediatric-inspired therapy does not improve young patient's outcome with high-risk Philadelphia-chromosome-negative acute lymphoblastic leukaemia. A single-centre report
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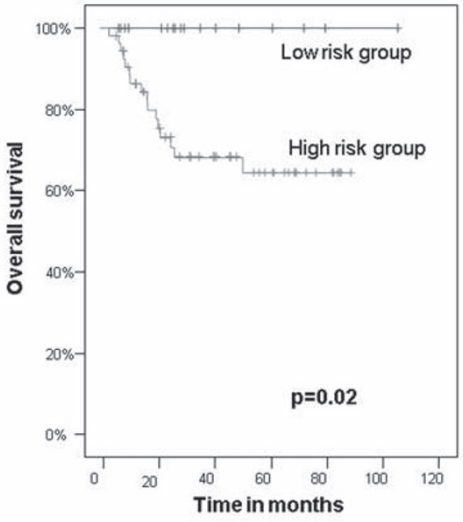
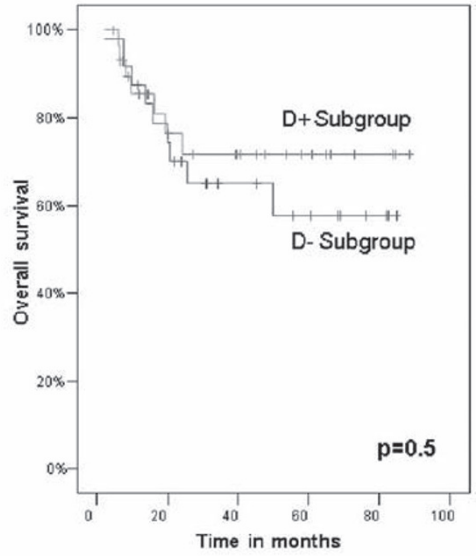
Allogeneic stem cells transplant (allo-SCT) is currently the preferred therapeutic option for young adults with Ph- ALL in first CR. However, the results of different studies suggested that pediatric-inspired therapy have markedly improved the outcome of these patients. In our monocentric study, we analyzed the impact of the allo-SCT on outcome in adults treated within these pediatric-inspired trials.
Between April 2002 and November 2010, 80 young adult (median age=33 years) with Ph- ALL were treated in our clinical unit. 74 patients were in complete remission (CR) after induction chemotherapy, 3 died before evaluation, 3 patients with refractory disease died. Among the 71 evaluable patients in CR, 54 were considered at high-risk ALL and therefore eligible for allo-SCT. Seventeen patients with low-risk ALL received chemotherapy alone with late intensification and maintenance therapy. With a median follow-up of 40 months, estimated OS for entire population at five years was 72%. In the low-risk (LR) group, none patient died but one patient relapsed. While, in the high-risk (HR) group, 13 patients relapsed and 16 patients died. For the LR group and the HR group, the estimated OS at five years was respectively 100% and 64% (p=0.02) (Figure 1) and the estimated disease free survival (DFS), 83% and 52% (p=0.03). In the HR group, 30 of the 54 patients with donor had received allogeneic SCT. The 24 patients without donor had received the same chemotherapy than patients in the LR group. There was no difference between the two subgroups for death: 7 patients with donor (D+) and 9 without donor (D-). Nevertheless, there was more relapses in the subgroup D- (n=10) than in the subgroup D+ (n=3) (p=0.02). At five years, in the subgroup D+, the estimated OS and DFS were respectively 72% and 63 %. In the subgroup D-, the estimated OS and DFS were respectively 58 % and 41 %. There was no difference between two subgroups D+ and D- for OS (p=0.5) (Figure 2) and DFS (p=0.16). There was no difference for characteristics of the disease.
These results suggest that allograft might not improve the outcome of patient with high-risk Ph- ALL. One explanation is that pediatric-inspired induction chemotherapy improves the outcome of the whole population and this advantage decreases the impact of the allo-SCT. Nevertheless, allo-SCT decreased the risk of relapse but did not modify OS and DFS. However, more patients are necessary to confirm these results in a multicentric study.





P922
Comparison of a fast transplant versus a classical conditioning strategy in allogeneic stem cell transplantation of high-risk acute myeloid leukaemia
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The aim of this analysis was to compare the fast transplant (FTx) to the well-established approach of performing allogeneic stem cell transplantation (SCT) in complete remission in high-risk acute myeloid leukemia (AML) patients (classical conditioning, CC). We retrospectively analysed 111 patients (pts) with high-risk AML who received a first allogeneic SCT in our center between January 2000 and December 2009. Sixty pts who had

achieved a complete remission (CR) at the time a donor with not more than one HLA mismatch was available were treated with a total body irradiation (TBI)-based or intravenous busulfan (iv Bu)-based conditioning regimens (CC). Fifty-one pts with active AML at the time of SCT received sequential treatment with intensive chemotherapy and reduced-intensity conditioning (RIC) as a FTx strategy. The FLAMSA-RIC protocol (fludarabine, cytarabine, amsacrine, cyclophosphamide, and TBI 4 Gy or iv Bu) was applied to 26 pts. Twenty-five pts were treated with a course of intensive therapy followed by fludarabine and melphalan.
Results: Patient age was significantly higher in the FTx than in the CC cohort (52 vs 46 years; p=0.017) and the interval from AML diagnosis or relapse to SCT was significantly shorter (76 vs 158 days, p<0.001). Both groups were comparable with respect to the proportion of pts with unfavorable cytogenetics (FTx 31 vs CC 40%), the use of unrelated donors (57 vs 65%) and peripheral blood stem cells (93 vs 88%). Estimated median overall survival (OS) of the entire cohort was 5.7 years (95% CI, 4.8-6.6 years). Patients treated with CC showed significantly superior OS rates of 82%, 72% and 67% at 1, 2, and 4 years after SCT compared to 64%, 50% and 33% in the FTx group (p=0.001), due primarily to a significantly lower incidence of non-relapse mortality (10 vs 25%, p=0.043). The incidences of relapses after SCT were not significantly different in both groups (23 vs 35%). Among the 51 pts of the FTx cohort, those transplanted with inadequate blast clearance or persistent AML after the first induction course (n=27) had a better outcome than the more heavily pretreated pts with relapsed disease (n=24). Median estimated OS in these groups was 4.0 vs 1.7 years and OS rates of 56 vs 33% at 2 years (p=0.02).
Summary: The fast transplant approach yields encouraging results regarding long-term survival in high-risk AML refractory to induction therapy, approaching the results of pts transplanted in complete remission using CC.

P923

Outcome of HSC transplantation for leukaemia after myeloablative conditioning using TBI (1,000cGy) delivered in 2 fractions: the Manchester Royal Infirmary, UK, experience

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Haemopoietic stem cell transplantation for patients with haematological malignancies at the Manchester Royal Infirmary began in 1983. For logistic reasons, we have used Total Body Irradiation (TBI) at a dose of 1,000 cGy delivered in 2 fractions as part of a myeloablative conditioning. This differs from the 1,200 cGy TBI given in 6 fractions commonly used in other centres. We report here our data from 139 patients (AML, ALL and CML) who were transplanted from a fully matched sibling donor. Together with TBI, AML and CML patients received Cyclophosphamide (120 mg/kg), while ALL patients received Cyclophosphamide (120 mg/kg) or Etoposide (60mg/kg). Cyclosporin ± Methotrexate was given for GVHD prophylaxis. Details of transplanted patients were as follows: ALL (n=29, 24 HPC-M and 5 HPC-A), median age 25 years, range 15– 46 years, AML (n=66, 43 HPC-M, 23 HPC-A), median age 37 years, range 18-54, CML (n= 44, 39 HPC-M, 5 HPC-A) median age 35 years, range 16-53. Acute leukaemia patients were transplanted in first CR and CML patients in chronic phase. The non-relapse mortality (NRM) at 100 days for ALL was 7%, AML 14% and CML 5% giving an overall mortality of 9%. NRM at one year was 10% for ALL, 21% for AML and 18% for CML, giving an overall mortality of 18%. Relapse risk at 3 years for ALL was 20%, AML 20% and CML 13%. For evaluable patients, acute graft versus host disease of grades 0 or 1 was seen in 72% with grade 2 or above in 28% (n=124) Chronic GVHD was absent or mild in 81% but moderate or severe in 19% of transplanted patients (n=79). Overall survival (derived from Kaplan-Meier plots) % at 1, 3, 5, 10 and 20 years post HSCT was as follows: ALL (n=29), 86, 76, 72, 65, 65%, AML (n=66), 74, 64, 59, 59, 50%, CML (n=44), 80, 73, 68, 68, 68% and total (n=139),79, 69, 64, 62 and 59% respectively.
Although a much larger number of patients have been transplanted, the data presented here is from the disease groups with the highest numbers to allow comparison with other published data. Our results compare very favourably with those reported by CIBMTR and EMBT registry for acute and chronic leukaemias and suggest that where necessary this 2 fraction TBI regimen can be considered as a suitable alternative to the conventional fractionated TBI commonly used in myeloablative allografts.

P924

Impact of the presence of an internal tandem duplicate of FIT3 receptor on the outcome of adult patients with acute myelocytic leukaemia autografted in first remsision (CR1)

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The prognosis of patients with AML harbouring the FIT3 ITD is known to be poor in patients treated with chemotherapy only. However it remains uncertain whether it also has an impact on outcome following high dose intensification and autologous stem cell transplantation (ASCT).
We adressed the question using the EBMT data base. Information on the presence or the absence of FIT3ITD was available in 318 adult patients with AML who were autografted in CR1 in the period of January 1999 to December 2009. 231 patients (115 with normal karyotypes) were ITD negative and 87 (34 with normal karyotypes) were ITD positive. 92% of the patients received peripheral blood as a source of stem cells.The follow up was 18 mo (1-117).
Patients ITD positive had higher white cell counts at diagnosis (55 vs 12.9 109/l ; p <0.0001) and more carried the NPM1 gene mutation (59% vs 37%, p=0.02).
For the 231 patients FIT3-ITD negative, the 3 year leukemia free survival (LFS) was 52 ± 3%, the relapse incidence (RI) 43 ± 4% and the non relapse mortality (NRM) 6 ±3%.
The 87 patients positive for Flt3ITD had a 3 year LFS of 36±6% and a RI of 58±7%.
When comparing the 4 groups of patients (FIT3ITD positive/negative and NPM1 positive/negative) , only the minority of patients with the FIT3ITD positive and NPM1 negative combination had a poor prognosis with a LFS of 19±11% (p=0.02) and a RI of 74± 15% (p=0.07).
Therefore the presence of a FIT3ITD /mutation is not an adverse prognostic factor for ASCT in CR1 if there is also a mutation of NPM1.

P925

Results of allogeneic haematopoietic stem cell transplantation in children and adolescents with acute myeloid leukaemia. Comparing efficacy myeloablative conditioning regimen versus reduced-intensity conditioning. Single-centre experience

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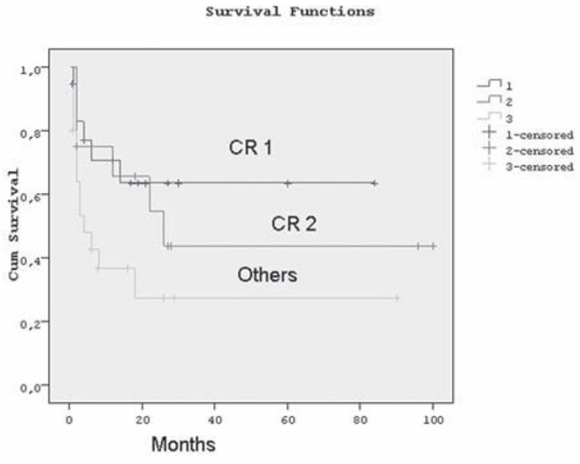
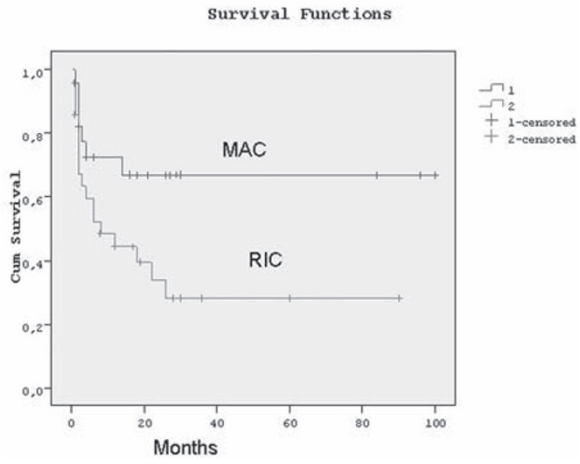
Background: The treatment of children and adolescents with acute myeloid leukemia (AML) has improved considerably over the past decades. It is connected with introduction more

intensive chemotherapy and using allogeniec haematopoietic stem cell transplantation (alloHSCT). However time of performing alloHSCT and efficacy of the usage different of conditioning regimen is controversial.
The aim of this study: To estimate results of alloHSCT in patients (pts) wih AML in different stage (1 complete remission (CR) high risk, 2 CR, other stages) exclusive of M3 FAB variant.
Patients: From January, 2002 to November, 2010 alloHSCT were performed in 53 pts (27 girls and 26 boys) with median age 11 years (range 2-20 y.o.). 19 pts were in 1 CR, 13 pts were in 2 CR, others were in progression disease or 3 CR (salvage groupe). 7 pts had secondary AML. Unrelated allo HSCT was done in 35 pts, related – in 18 pts. Source of HSCT: bone marrow (BM) in 20 pts, peripheral blood stem cells (PBSC) in 33 pts. Median CD34+ - 6,8*106/kg. Mieloablative conditioning regimen (MAC) was used in 25 pts, reduced intensity conditioning (RIC) in 28 pts. MAC consisted Busulfan 16 mg/kg + Cyclophosphamide 120 mg/kg. RIC included Fludarabine 150 mg/m2 + Melphalan 140 mg/m2 in 12 pts, Fludarabine 150 mg/m2 + Busulfan 8 mg/kg in 15 pts, FLAG in 1 pt. Acute graft versus host disease (aGvHD) prophylaxis was by Cyclosporine (CsA) and short course of MTX in 38 pts, CsA +MMF in 6 pts, Tacrolimus and MMF–9 pts. 2 pts after related allo HSCT and all pts after unrelated HSCT recieved ATG 60 mg/kg.
Results: Engraftment in most pts was at day + 17 (range 10-30). Primary non engraftment was 7% (3 pts). 2- years overall survival (OS) in all group was 52 %. OS in pts with 1 CR was 68%, with 2 CR – 50%, others has OS 35% (Picture 1). OS in pts after MAC was 70%, after RIC 35% (Picture 2). MAC demonstrated advantage versus RIC in groupe with CR1 and CR 2: OS was 72% vs 50%; and in salvage group: OS 67% vs 16% (p>0.05), respectively. We did not reveal significant difference in OS between children and adolescents: 55% vs 47%; between BM and PBSC: 58% vs 46%; between related or unrelated donor: 50% vs 52%, respectively. The worst OS was in pts with secondary AML- 15%.
Conclusion: allo HSCT is effective in children and adolescents with AML, the best results in OS observed in pts with CR1, after MAC. So allo HSCT seems to be the treatment of choice in pts having suitable donor (related or unrelated).





P926

Autologous stem cell transplantation in AML in first CR. A retrospective single-centre experience of uniformly treated patients

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Although >80% of AML patients age 60 and younger achieve CR, substantial percentage will relapse. Even among the favorable cytogenetics, there is a wide spectrum of published results using standard post remission therapy. One of the largest reports in 370 Patients (Appelbaum FR et al BJH, 2006) soberingly reported a long term survival of only 44 %. Post remission therapy in AML is largely dictated by cytogenetic and molecular risk groups. Autologous stem cell transplantation (ASCT) in AML is controversial due to lack prospective studies in the era of peripheral stem cell harvesting, intent to treat analysis while many have not received the assigned treatment and a high TRM. However, the preponderance of prospective studies report a potent anti-leukemic advantage for ASCT in terms of reduced relapse and superior DFS albeit no OS advantage. The current study retrospectively evaluated a large cohort of uniformly treated patients undergoing ASCT. Methods: 115 consecutive patients were included in the analysis. Between 1996-2001: ASCT was performed in young adults if no matched related donor (MRD) or age>55. Between 2001-2010: ASCT if: 1. Favorable cytogenetics. 2. Intermediate & high risk with no MRD or age > 65. Median age 47, 107 in CR1 after standard 7+3 followed by HIDAC X2, and 8 with APL in CR2. Conditioning regimen was busulphan/cytoxan in 90.4%. All patients received peripheral blood stem cells. With a median follow-up of 3 years (0.5-14) cumulative OS is 52.24%. Mean OS: 8.5 years, EFS rate: 48.76% with a mean of 7.47 years, relapse rate: 46% and TRM of 0.89%. OS for favorable, intermediate and high risk was 52.9%, 57.9% and 41% respectively. EFS was 55.490%, 56.09% and 28.09%, respectively. In multivariate analysis: favorable and intermediate risk groups had superior OS (p=0.015, HR 2.8), and a trend for improved EFS (p=0.055, HR=2.07). Age < or ≥ 60 and daunorubicin dose were not associated with difference in outcome.
Conclusion: With a long term follow up outcome of favorable and intermidiate risk groups was comparable with significante OS and a trend for improved EFS compared to high risk group all with low TRM. These data suggest that (a) randomized studies should asses the role of ASCT in the era of current practice, and (b) although based on relatively small numbers, the data suggest that autologous transplant may also be an option for patients with intermediate and unfavorable cytogenetics who do not have a donor.

P927

Results of second allogeneic stem cell transplantation for children and adolescents with acute leukaemia

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The aim: This analysis was performed to asses overall survival (OS) of children and adolescents with acute leukemia (AL) after

second allo-HSCT (2nd allo-HSCT) and reveal risk factors for OS (to identify factors which have a prognostic significance).
Patients and transplant: 186 patients (pts) from 1 till 21 y.o. (mediana 12 y.o.) were underwent allo-HSCT between 12/2000 and 11/2010. A second allograft was performed in 22 of these pts. Interval from first HSCT to second HSCT was 1-23 months: in 16 pts ≤ 6 months, in 6 pts > 6 months. The cause of 2nd allo-HSCT was disease recurrence after 1st allo-HSCT in 21 pts and non-engrafted in 1 patient. In 22 patients with 2nd allo-HSCT diagnosis was ALL (n=12) and AML (n=10). Status disease at the moment of 1st allo-HSCT was 1 or 2 complete remission in 7 pts (32%), relapse or resistance in 15 pts (68%). Ten patients were transplanted from a matched sibling donor (MSD), 4 pts from matched unrelated (MUD) and 8 pts from an alternative family donor (Haplo). In 14 cases 2nd allo-HSCT was performed using the same donor as in 1st allo-HSCT. RIC 1st allo-HSCT was performed in 9 pts. MAC was used in 13 pts. (RIC consisted of Flu 150 mg/m2/d + Mel (140 mg/m2/d)±ATG or Flu 150 mg/m2/d + Bu 8 mg/kg±ATG; MAC consisted of Bu 16 mg/kg (or Treo 36-48 mg/m2) +Cy 120 mg/kg ±ATG). Al 2nd allo-HSCT was performed with RIC.
Results (tab 1): OS (3-y) of children and adolescents with acute leukemia (AL) after 2nd allo-HSCT was 17%.
The table 1 shows comparative analysis of OS of pts from different groups. The statistically significant parameters associated with 3-years OS were the following: status at 1st allo-HSCT, type of donor and interval from 1st allo-HSCT to 2nd allo-HSCT. The choice of donor (same or other), intensity of conditioning (RIC or MAC), age at the moment of allo-HSCT (under 14 y.o., after14 y.o.), type of AL (ALL or AML) didn't have significant influence on OS.
Conclusion: Second allo-HSCT is feasible and effective in pts that have relapse > 6 months after 1st allo-HSCT or transplantation at the 1st allo-HSCT in 1or 2 CR.

Parameter		3y OS	p value
Interval from 1st allo-HSCT to 2nd allo-HSCT	≤6 months >6 months	10% 33%	0,04
Status at 1st allo-HSCT	1,2 CR Relapse or resistance	38% 8%	0,01
Type of donor	MUD MSD Haplo	33% 25% 0	0,04
Choice of donor	Same other	18% 19%	0,539
Age at the moment of allo-HSCT	Under 14 y.o. After 14 y.o.	37% 14%	0,307
Diagnosis	ALL AML	10% 25%	0,441
Conditioning	RIC MAC	15% 19%	0,904

P928

Once-daily intravenous busulfan and fludarabine as conditioning for patients with significant co-morbidities and high-risk myeloid disease undergoing allogeneic haematopoietic stem cell transplantation is well tolerated and very effective

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Objective: To analyze the efficacy, based on toxicity and outcome, of intravenous (IV) busulfan(BU) and fludarabine(FLU) as conditioning regimen of allo-HSCT in patients with myeloid diseases.
Patients and methods:62 patients underwent allo-HSCT in our hospital from May-2006 to Sept-2010.Conditioning regimen consisted of BU (3,2 mg/Kg, IV) and FLU (40 mg/m^2, IV) both given once daily for 4 days. Median age was 48 years (24-74) and 29 patients were male. Myeloid diseases were: 44 AML[48% considered as high risk (secondary-AML, adverse genetics)], 13 MDS(8 with IPSS intermediate-2/high) and 5 chronic-MPD. Disease status at allo-HSCT was 1CR: 37, 2CR: 6, >3CR/refractory disease: 4, stable disease/PR:15. Donor were: matched related (30), matched unrelated (19), mismatched (13) and stem cell source was bone marrow in 92%.Cyclosporine or tacrolimus combined with methotrexate or mycophenolate was used as GvHD prophylaxis and only 5 patients (8%) received ATG. Based on Sorror HCT-CI, patients were divided into low risk (score 0:29%), moderate risk (score 1-2:34%) and high risk (score>3:37%).
Results: Median time to PMN (>500/mm3) and platelet recovery (>50.000/mm3) was 16 days (11-28) and 16 days (11-135). Complete chimera at day +30 was achieved in 83% of patients. Only 3 patients (5%) had graft failure (primary 1 and secondary 2). 1 patient (2%) had a reversible hepatic veno-occlusive disease and 40 patients (65%) developed moderate-severe mucositis. The rates of grade II-IV and III/IV acute GvHD were 35% and 8%. aGVHD was more frequently in unrelated donor (64% versus 36%, p=0,009). Three patients (5%) developed corticosteroid refractory aGvHD. Thirty four patients developed chronic GvHD that was extensive in 16. Early transplant-related mortality (TRM <100 days) was 8,1% (2 leukaemic progresion, 2 infection, 1 refractory aGvHD). Three-year estimated overall survival (OS) and event free survival (EFS) were 58% and two years OS was 79% for patients in 1CR. OS in patients with matched-related, matched-unrelated and mismatched donor were 67%, 78% and 44% respectively. According to HCT-CI, OS in low and intermediate/high risk was 79% and 49%. Three years OS in AML, MDS, and chronic-MPD were 62%, 61% and 100%.
Conclusion: BUFLU conditioning for patients with significant comorbidities and high-risk myeloid disease undergoing an allo-HSCT is well tolerated and very effective with a low early TRM and aGvHD incidence and with EFS that compare favourably with other conditioning regimens.

P929

Allogeneic stem cell transplantation for patients with AML and FLT-3 mutations

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Patients with acute myeloid leukemia (AML) with FLT-3 mutations have an extremely high risk of relapse after conventional chemotherapy. The role of allogeneic stem cell transplantation (SCT) for this patient cohort has been discussed controversially in recent years. This report summarizes our cumulative experience in a cohort of 42 consecutive patients (age 17-70, median 51 years) allografted for FLT-3 positive AML in a single

centre. In more than 80% a FLAMSA-RIC based conditioning regimen was used, in 5% BCNU/Melphalan/Fludarabine, and in 14% conventional radiation- or Busulfan- based regimens. Most patients received mobilized peripheral blood stem cells as graft and 10 patients had a sibling and 32 an unrelated donor (MUD), respectively. Half of the patients were allografted in complete remission and twenty with active, mostly refractory disease. With a median follow-up for surviving patients of nearly 2 years (range 64 – 1746 days) the Kaplan-Meyer procedure estimates a 48% probability of survival at 2 y after transplantation. Interestingly, there is no difference what so ever in survival if patients had an identical sibling donor or a MUD. Similarly, neither patient age below or above the median, nor the applied conditioning regimen did affect the outcome. The only significant variable for improved survival was being in complete remission at transplantation with a 2-year overall survival probability of 60% as compared to 30% for patients with active disease. Thirteen patients (31%) relapsed after allografting, which is substantially lower as to what is reported after conventional chemotherapy. Three of these patients could be salvaged by a second transplant, whereas 10 patients finally died from leukaemia. Non relapse mortality was 24% with 2 patients dying from acute GVHD, 7 from infections and 1 from suicide, despite being well physically. In conclusion, our data support the notion that allogeneic SCT is a highly effective treatment option for patients with AML and FLT-3 mutations and that, if the patient is eligible, it should be undertaken whenever possible in 1. complete remission. However, even patients with primary induction failure have a reasonable chance to be salvaged by allogeneic SCT.

P930

Outcome of allogeneic haematopoietic cell transplantation for acute myelogenous leukaemia: a single-centre retrospective analysis

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The selection of patients (pts) upon the best stratification and the timing of transplantation are the key issues of clinical management in AML. We analyzed the data of all pts (149) allografted consecutively for AML in our BMT-unit during 1991-2009. Donors were siblings (107), relatives (11), unrelated (25), double cord blood CB (1), haploidentical (5). Sixty-three pts aged 35(8-63) were transplanted in CR1 after myeloablative (MA) (56) and non- myeloablative (NMA) regimen (7). Peripheral blood (PB) was the main graft source (51). Karyotype was available in 40 pts (intermediate 32, poor risk 8). Eighty-six pts were allografted beyond CR1: 42 Prim.Ref, 15 CR2, 23 Rel1 and 6 advanced (CR3; Rel2+). The majority of pts received PB (72) and MA regimen (82). Karyotype was available in 71 (favourable 4, intermediate 53, poor 14). For CR1 pts OS was 63%, NRM 23%, DFS 60% and RR 21% at 13 years, whereas for 46 pts transplanted after 1999 NRM was lower (17% at 9 years). DFS for CR1 pts with unrelated donor was 47% and 62% for siblings. The outcome post NMA was poor (DFS 21% vs 65% post MA). According to cytogenetics OS and DFS were 62% and 64% for the intermediate, 44% and 45% for poor risk respectively. For CR2 pts OS was 51% and DFS 46%, RR 43% and NRM 16%. For Prim.Ref. pts OS was 20%, DFS 17% (plateau at 2 years) and NRM 34% at 12 years. Poor risk karyotype pts (7) had dismal outcome (DFS, OS 0%) vs 25% and 31% respectively for the intermediate risk group (28). For pts in REL1 OS was 15%, NRM 56%, DFS 4% and RR 86%. For the 6 pts transplanted for advanced disease OS/DFS was 17% and RR, NRM rates high. The 5 pts undergone haploidentical hematopoietic cell transplantation (HCT) had OS and DFS 40% at 8 years. In multivariate analysis significant factors associated with better outcome were early phase disease (p=0.0001), lower risk karyotype (p=0.04), presence of acute graft versus host disease (GVHD) and chronic GvHD (p=0.047, p=0.01), while PB had a borderline significance (p=0.08). Factors associated with lower NRM were the time of transplantation (>2005, p=0.001) and identical-sibling donor. According to our experience, allogeneic-HCT for AML in early phase seems to have the potential to cure a significant proportion of pts with low NRM, even in the alternative setting, with improving results during the late time frame. In contrast, a small proportion of refractory pts may be rescued by HCT, specifically in the intermediate cytogenetic risk group.

P931

Improved outcome in patients with acute myelogenous leukaemia post high-dose chemotherapy with the combination of BCNU, cytarabine, melphalan and autologous haematopoietic cell transplantation

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Autologous hematopoietic cell transplantation for selected patients (pts) with acute myelogenous leukemia is a viable and reliable option of therapy, even in the era of alternative or reduced intensity transplants. The main advantage of the procedure is very low toxicity and treatment related mortality rate (TRM). We retrospectively studied 51 pts, aged 37 (12-54) years with de novo AML (49) and secondary AML (2) autotransplanted in CR1. The conditioning regimens used were: a)BEAM modified (marrow harvest on day -3, infusion of BCNU day -3, Etoposide and Cytarabine day -2, Melphalan day -1) and fresh marrow graft (20), b) BUCY-2 (10), c) BUCY-4 (6) and d) BCNU-AraC-Mel (BCNU 300 mg/m² day -3, Cytarabine 3 gr/m²/12 hours day -2, Melphalan 140 mg/m² day -1) (15) with cryopreserved graft either marrow (14) or peripheral blood (PB) (17). The PB graft was mobilized post high dose Etoposide (1,6gr/m²) and has been used in the recent period 2000-2009. For the whole cohort of 51 pts auto-transplanted form 1987-2009 the probability of DFS and OS was 28% and 33% respectively with a Dm follow-up 15 (9-20) years and TRM 6%. In terms of the time of transplant for the early period (1987-1999) the DFS rate was 27%, relapse rate 57% and TRM 10% at 21 years. On the contrary, the OS and DFS of pts who received the BAM regimen and mobilized PB as graft during the late period 2000-2009 was 53% and 83% respectively, while the TRM was 0% at 8 years. Interestingly, pts with intermediate risk group cytogenetic (17), mostly normal karyotype, succeeded rates of OS 82% and DFS 57% at 8 years. The challenge of refinements of clinical management of patients with AML is to identify individual patients likely to benefit from specific therapeutic modalities, such as autologous hematopoietic cell transplantation, as an intensified consolidation treatment with zero morality rate. It can be employed to maintain the remission and cure the disease. Better supportive care has enhanced the ability of nearly each AML patient to deliver high dose chemotherapy plus autologous rescue with mobilized PB graft. Moreover, comparison of various used conditioning regimens demonstrated a DFS survival advantage of BAM (49%) over the others, ie modified BEAM (32%) or BUCY-2, BUCY-4 (31%) at 7, 21 and 18 years respectively.

P932

Is there an impact of KIR and KIR/ligand genetic polymorphisms in acute lymphoblastic leukaemia development?

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Associations of specific KIR and/or KIR/HLA genotypes with certain diseases have been reported but the relevance of KIR and KIR/ligand polymorphisms on the development of haematological malignancies is still less clear. The objective of this study was to investigate KIRs, their HLA class I ligands and KIR/ligand combinations in case-control as well as in family-based study, and to assess if particular gene profiles mediating inhibitory or activating NK cell function may be associated with acute lymphoblastic leukemia (ALL) development. Thirty patients with ALL treated in Children Oncohematology Hospital and 93 non affected members of their families, and 80 unrelated randomly selected healthy controls from the Bulgarian population were evaluated for KIR and KIR HLA ligand gene polymorphisms by PCR-SSP method (Olerup SSPTM KIR and KIR HLA Ligand Kits). KIR2DS1 frequency was lower in ALL patients (36.7% vs 50%) while the incidence of KIR2DS3 was higher in healthy related individuals (52.7% vs 37.5%; p<0.05) compared to randomly selected controls. No significant differences were found in KIR A and B haplotype distribution between the compared groups. Homozygosity for HLA-Cw group 1 (HLA-Cw alleles with asparagine at position 80) and HLA-A alleles with Bw4 motif which serve as ligands for certain KIRs, were more frequent in ALL patients compared to unrelated controls (43.3% v.s. 26.2%, and 46.7% v.s. 28.8%, respectively). KIR3DL+/BBw4+ABw4- combinations were lower in patients (30%; p<0.05) and their siblings (24.3%; p<0.01) in comparison to randomly selected healthy controls (51.2%). Moreover, a trend of higher incidence of individuals bearing both ligands for inhibitory KIR3DL1 (3DL1+/BBw4+ABw4+) was observed among patients (26.7%) and their healthy relatives (33.3%) compared to unrelated controls (16.25%). These data support the involvement of HLA-C locus and HLA-A Bw4 alleles in modulating the risk of ALL perhaps through their function as ligands for KIRs. In addition, the activating genotype KIR2DS1+/C2+ was less common in patients in comparison to unrelated controls (33.3% vs 13.7%; p<0.05). Our data suggest that inherited genetic polymorphisms within immune response genes may lead to a domination of inhibitory over activating KIR/ligand signals, which contributes to the escape of emerging malignant cells from immune surveillance and development of ALL. These findings may also have impact in leukemia treatment using NK cell alloreactivity.

P933

Outcome of autologous haematopoietic stem cell transplantation in adults with acute myeloid leukaemia using three different conditioning regimens

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The role of autologous Hematopoietic Stem Cell Transplantation (HSCT) in acute myeloid leukemia (AML) remains controversial. Our HSCT Program adopted a policy to perform autologous hematopoietic stem cell transplantation for AML patients in =/> 1st complete remission (CR1) who had no HLA-matched related donor and who mostly fall in the intermediate or high risk group.

Methods: This is a retrospective analysis of the King Faisal Specialist Hospital and Research Center HSCT registry data base for all AML patients who underwent autologous HSCT between 1985-2009. Variables examined included age, sex, disease risk group, disease status at transplantation, conditioning regimen and source of stem cells. Kaplan and Meier method was used to estimate disease free survival (DFS) and overall survival (OS). Groups were compared using the log-rank test.

Results: 113 autologous HSCT procedures were consecutively performed during this period. Median age was 32 yr (range 14-60). There were 65 males & 48 females. Median follow up is 20 (1-255) months. Disease status at transplant: CR1 (n=70, 62%) & =/>CR2 (n=43, 38%). Three different conditioning regiments were used: CY/TBI, BU/CY, and BU/VP16. Most of the patients transplanted after the year 2000 were in the BU/VP16 (n=50, 44.2%) group while earlier cohorts were split between CY/TBI (n=39, 34.5%) and BU/CY (n=24, 21.2%) conditioning. Source of stem cells: bone marrow (n=37, 32.7%, mostly in the earlier cohort of patients), peripheral blood (n=70, 61.9%) and BM+PB (n=6, 5.3%). All patients in the BU/VP16 (later cohort) group received peripheral blood stem cell. Five year OS & DFS for the whole group were 45.6% and 43.2%, respectively. A trend of improved outcome using BU/VP16 conditioning (OS 60.3%, DFS 50%) was observed but this was not statistically significant (p value= 0.1 and 0.3, respectively; Figure 1 and 2). Disease status at transplant showed a trend of better outcome in CR1 (OS 51%, DFS 47.4%) compared with =/>CR2 (OS 37.2%, DFS 36%) without statistical significance (p value = 0.5 and 0.6 respectively). Procedure related mortality for the whole group was 6.2 %.

Conclusion: Autologous HSCT is a reasonable and relatively safe therapeutic modality for patients with AML who have no suitable related donor, with outcomes comparable to those reported using matched unrelated or cord blood transplantation.

Figure: 1

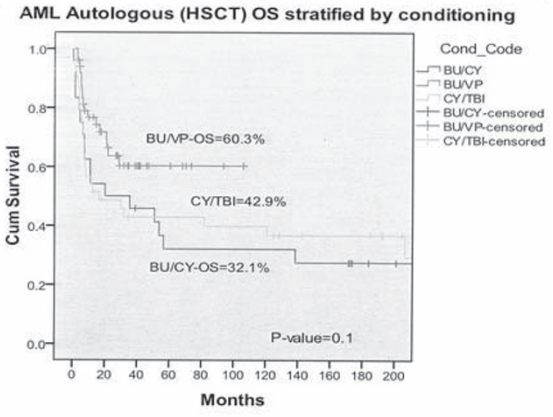
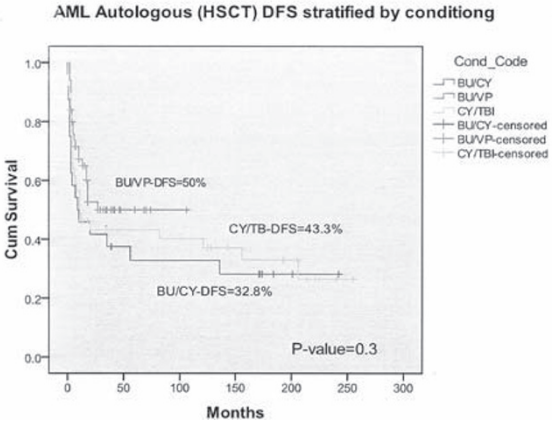


Figure: 2



P934

Peripheral and bone marrow blast cells, cytogenetic pattern and donor type are relevant factors for the outcome of allogeneic stem cells transplant in high-risk acute myeloid leukaemia

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The aim of this study was to analyze the outcome and to identify prognostic factors in 114 consecutive AML patients (pts) submitted to HSCT in second/greater complete remission (CR) or in active disease at our Institution between 1993 and 2010. They were 53 females and 61 males with a median age of 43.8 years (range 18.6-66.7). At the time of transplant 41 pts (36%) were in second/greater CR, 28 (25%) in partial response or in first initial relapse and 45 (39%) had refractory disease. Considering pts not in remission, three, in molecular relapse, had a bone marrow (BM) blast cell percentage less than 5, twenty-eight comprised between 5 and 20 and thirty-four over 20. BM was not performed in 8 pts, included in the 39 with peripheral blast cells. At diagnosis cytogenetic analyses were successful in 107 pts, 29% considered good risk, 44% intermediate risk and 27% high-risk (HR). The donor was matched related/unrelated in 43 and 55 pts and haploidentical in two. One pts received a cord blood transplant. Sixty-five pts (57%) received peripheral stem cells, 48 (42%) from BM stem cells. Conditioning was myelo-ablative in 73% of patients and reduced in 27%. Five-yrs OS was 38% and the 5-yrs cumulative incidence of transplant-related mortality (TRM) and Relapse Rate (RR), estimated with a competing risk approach, were 37% and 28%. The 5-years OS, TRM and RR were 43%, 21% and 36% for sibling versus 34%, 47%, 15% for unrelated transplants (all p values<0.03 by Wilcoxon test). The 5-years TRM and RR were 30% and 15% for pts in CR versus 40% and 34% for those transplanted with active disease (p=ns and p=0.07). Cytogenetic high-risk groups had a relevant influence on OS (p=0.003). The 5-years OS was 49% for good-risk patients, 44% for intermediate-risk patients and 14% for HR patients. When multivariate Cox proportional-hazards regression was applied, BM blasts percentage >20% and unrelated donor had a significant negative influence on OS (p=0.013 and p=0.001 respectively) and TRM (p=0.01 and p=0.001); peripheral blood blasts percentage and reduced intensity conditioning negatively affected RR (p=0.006 and p=0.03). The cytogenetic pattern showed a trend toward significance for OS (p=0.05) and had a significant influence on RR (p=0.01). In conclusion, BM blast cell percentage and donor type are the most relevant factors for OS and TRM, whereas blast cells in peripheral blood, cytogenetic pattern and conditioning regimen the most relevant for RR.

P935

Retrospective analysis of clofarabine/Ara-C salvage chemotherapy preceding allogeneic stem cell transplantation for AML

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Objectives: Overall prognosis of acute myeloid leukemia (AML) refractory to induction chemotherapy remains extremely poor. As the only curative option, allogeneic stem cell transplantation (SCT) may provide immunological protection from relapse by delivering beneficial graft-versus-leukemia effects. Prerequisite for such a successful transplantation is a situation of minimal residual disease or minimal tumor burden. Clofarabine has strong anti-leukemic activity but is associated with long-lasting aplastic periods. To this end, we investigated a clofarabine/cytarabine salvage chemotherapy followed by allogeneic SCT in order to restore hematopoiesis and to enable graft versus leukemia activity after effective eradication of leukemic blasts for AML patients that failed induction chemotherapy.
Methods: We retrospectively reviewed data from all 32 patients available for analysis with refractory or relapsed AML that were treated with 5x40 mg/m² clofarabine between March 2007 and November 2010 in our institution.
Results: After salvage chemotherapy with 5x40 mg/m² clofarabine and cytarabine we observed clearance of leukemic blasts in 69% (22/32) of our patients. However, in those not achieving a complete clearance of their disease a substantial reduction of leukemia burden could be documented (7/32). Blast persistence or progressive disease was limited to 3 patients only (9%). Twenty-three patients proceeded to allogeneic SCT using HLA matched (70%), haploidentical (27%) and 1 double cord blood graft while nine patients died before transplantation. After a median follow-up of 173 days (range 1-1013 days), overall survival was 48% (11/23) of all allografted patients.
Conclusion: The combination of clofarabine and cytarabine preceding allogeneic SCT combines the excellent tumoricidal activity of clofarabine with the restoration of hematopoiesis of the allogeneic graft. Clofarabine and cytarabine has an acceptable toxicity profile, compares favorably to other published salvage strategies and does not negatively interfere with subsequent allogeneic SCT. Taken together, the data provided here warrant further clinical trials that prospectively investigate the effectiveness of clofarabine salvage chemotherapy prior to allogeneic SCT for refractory AML.

P936

In haematopoietic SCT recipients, pre- and post-transplant cytomorphologic remission status has strong prognostic relevance, but no predictive value during reduced-conditioning phase in AML patients

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Patients (pts) with acute myeloid leukemia (AML) who received hematopoietic stem cell transplantation (HSCT) undergo periodic surveillance through analysis of bone marrow (BM) cytomorphology. Only few studies evaluated the prognostic relevance of the remission status in AML pts after HSCT, and no study analyzed the predictive value of early blast clearance during reduced intensity conditioning (RIC) phase. Here, we report the correlation between survival at 24 months and the BM blast population at different times before and after HSCT.
We analyzed data from 104 pts (73 de novo AML, 27 s-AML/MDS, 4 t-AML) treated in our center between 2005 and 2008: median age 51 yrs (range 19-72). Pts were treated with RIC (n=69) or myeloablative conditioning (MAC) (n=35) for HSCT. Remission status before HSCT was induction failure or recurrent disease in 61 pts, 1st complete remission (CR1) in 24 pts, >CR2 in 19 pts. The RIC regimen was FLAMSA (fludarabine, high dose cytarabine, amsacrine) from days -10 to -7 followed by busulfan and antithymocyte globulin (ATG) from days -3 to -1 to HSCT. Only cases with available BM cytomorphology before (n=104) and after HSCT (day 30: n=95; day 100: n=77) were included. In RIC pts, additional analysis of cytomorphology was performed in the interval between FLAMSA and application of busulfan/ATG (n=51).
After a median follow up of 24 months (range 0.1 to 51.5), the overall survival (OS) was 39.4% (RIC 26.1%, MAC 65.71%) and leukemia-free-survival (LFS) was 45.2% (RIC 21.7%, MAC 60%). We identified that blast percentage in BM specimens (<5% vs >5%) before HSCT correlate with survival (both OS and LFS) at 24 months (OS 73.6% vs 30%, p <0.001; LFS 66% vs 25%, p <0.001). Likewise, blast percentage (<5% vs >5%) after HSCT measured at days 30 and 100, correlate with survival (OS) at 24 months (day 30: OS 52% vs 0%, p=0.001; day 100: OS 71.6% vs 20%, p < 0.001). We found no significant correlation between blasts <5% (n=17) vs >5% (n=34) in BM specimens during RIC conditioning phase and survival measures: mean OS was 232 vs. 248 days.

Conclusion: Cytomorphology before and after HSCT is a potent predictor in AML but early blast clearance during FLAMSA-RIC conditioning phase allows no prediction of LFS or OS. Hence, this parameter is not effective to identify specific risk groups who might benefit from intensified surveillance programs or modified immunotherapeutic strategies, what emphasizes the need of alternative monitoring strategies.

P937

Treatment outcome of adolescents and young adults with acute lymphoblastic leukaemia: a single-centre experience

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Background: Acute lymphoblastic leukemia (ALL) is a heterogeneous disease and outcomes vary by patient age, immunophenotype and clinical, cytogenetic and molecular features. 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 patients (pts) have a better outcome when treated with pediatric, rather than adult therapeutic protocols.
Aim: The purpose of our study was to analyse the treatment outcome in adolescents and young adults aged 15 to 30 years with newly diagnosed ALL who were treated in our adult hematology department.
Patients and methods: A total of 73 pts, male/female 50/23, aged 15 to 30 (average age 21), diagnosed with ALL during 1989-2009 in our institution were treated with adult YU-ALL protocol. Pts were divided in two subgroups according to disease risk parameters (standard/ high risk), age (15-18 vs 19-30), white blood count (> 30x109/l vs < 30x109/l), time to achieve remission (below 28 days vs above 28 days). Also, they were divided considering to postremission treatment (conventional maintenance therapy – 36 pts vs stem cell transplantation SCT– 37 pts). Twenty six pts were underwent allogeneic SCT from identical sibling donor and in 11 pts autologous SCT was performed. Majority of pts treated with allogeneic SCT had initially „high“ risk of the disease.
Results: For the whole cohort, the remission rate was 90,4%, iduction failure 9,6%, early deaths 3,2% and relapse rate 57,5%. After a median observation time of 7 years the overall survival (OS) and event-free survival (EFS) of pts who were treated with allogeneic SCT were superior in comparison with pts who were treated with conventional therapy (OS 42% vs 27%, $p < 0.05$; DFS 57% vs. 31%, $p < 0.05$). Relapse incidence was significantly lower in the group of pts with allogeneic SCT (32% vs 69%, $p < 0.05$). 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: Our results of treatment adolescents and young adults with adult therapeutic regimens are inferior to results of other study groups who have used pediatric protocols for same pts group. Allogeneic SCT is still the best treatment option for sellected number of pts with ALL, but further investigation, especially in the new target agents era is needed.

P938

Early induction of complete remission is predictive for outcome in refractory AML patients treated with FLAMSA-RIC

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Purpose: Allogeneic stem cell transplantation (SCT) is the only curative treatment for patients with refractory acute myeloid leukemia (AML). The outcome, however is still poor. We analysed our experience of patients with resistant disease transplanted using a sequential regimen of chemotherapy and reduced intensity conditioning allogeneic SCT (FLAMSA-RIC).
Patients and methods: We retrospectively analysed 22 patients with a median age of 40 (range 20-64) years and refractory AML. Seven patients had primary induction failure (PIF), ten patients had early relapse after first complete remission (CR) and five patients late or second relapse. Patients underwent SCT after a median of 3 (range 2-8) lines of chemotherapy and leukemic blasts of median 17 (range 6-80) %. All were transplanted after a chemotherapy consisting of fludarabine (4x30mg/m²), cytarabine (4x2 g/m²) and amsacrine (4x100 mg/m²) followed 4 days later by 4 Gy total body irradiation, cyclophosphamide (120 mg/m²) and antithymocytic globulin (6 mg/kg). Patients without graft-versus-host-disease (GvHD) at day +120 received donor lymphocyte infusion (DLI).
Results: With a median follow up of 10 (range 2-39) months, nine of the 22 patients (41%) were alive. CR was observed on day 28 after SCT in 42% of all patients, while 53% had 5-20% blasts (PR). Half of the patients with PR converted into CR by day +56 after reduction of immunosuppressive therapy resulting in an overall CR rate of 68%. Estimated overall survival (OS) and relapse incidence (RI) at two years were 27% and 75%, respectively. Patients with < 20% bone marrow infiltration prior to FLAMSA-RIC had a significantly better OS than patients with ≥ 20% blasts (66% vs 17% after one year, $p=0.01$). Patients with a maximum of two lines of therapy before allogeneic SCT had a trend towards better OS, while patients with PIF had a better LFS than patients with early relapse. Patients with grade 2 acute GvHD had the best OS of 67% after 2 years ($p=0.045$) and those receiving DLI (n=5) had a significantly lower RI of 20% resulting in a better LFS ($p=0.01$).
Conclusion: FLAMSA-RIC results in a high CR-rate in refractory AML patients. The main factors influencing outcome were blast count prior to SCT, lines of pre-treatment, grade of acute GvHD and the administration of DLI. Early reduction of immunosuppression is an important factor to prevent relapse in this cohort of patients.

P939

Genome-wide profiling of structural genomic variations in Korean AML patients

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Structural genomic variations are of predominant importance to the biology and clinical outcome of patients with acute myeloid leukemia (AML), and conventional karyotype-based risk classifications are routinely used in clinical decision making in AML. However, almost half of AML patients have normal karyotype, and this subgroup shows a various response to therapy. Recent advances in genome-wide single-nucleotide polymorphism (SNP) analyses have revealed previously unrecognized microdeletions and uniparental disomy (UPD) in a broad spectrum of human cancers. As AML represents a genetically heterogeneous disease, this technology might prove helpful, especially for cytogenetically normal AML patients. Here we have analyzed 54 AML blast-derived DNAs with normal karyotypes and 200 healthy controls using Illumina 317K SNP arrays. Chromosome aberrations (> 2 Mb) were more frequently observed in AML patients (30%) than healthy controls (4%). Regions of UPDs were identified in 26% of patients and chromosome 13 was most frequently affected. Average length of aberrations per individual was also larger in AML patients (14.9 Mb) than healthy controls (0.2 Mb). The numbers of detected copy number variations (CNVs, < 2 Mb) were 4,438 and 2,066 in AML patients and healthy controls, respectively. Average number and size of CNVs per individual were greater in AML patients (82.2 CNVs and 101.2 Kb) than healthy controls (10.3 CNVs and 65.1 Kb). In further analysis, we showed that the frequency and/or

average size of chromosome aberrations/CNVs were associated with complete remission attainment in AML patients. These data show the potential of high-resolution SNP analysis for identifying genomic regions of potential pathogenic and clinical relevance in AML.

P940

Treatment outcome in AML according to cytogenetic and molecular genetic risk grouping; single-centre experience

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Background: The European LeukemiaNet has lately given a recommendation for correlating cytogenetic and molecular genetic data with clinical data in AML (Döhner et al. Blood 2010). According to this recommendation, the purpose of this study was to retrospectively analyse the outcome of younger patients with non-APL AML treated at Turku University Hospital from Jan 2000 to Jul 2010.
Methods and results: During the 10-year period 93 patients aged 18-65 years with de novo or secondary AML were treated within the AML protocols of the Finnish Leukaemia Group. Patients were stratified into 3 risk groups according to baseline cytogenetic and molecular genetic findings (NPM1-mutation and FLT3-ITD) as indicated by the European LeukemiaNet. Thus, favourable, intermediate, and adverse risk groups included 24, 39, and 30 patients, respectively. CR rate after the first induction was 73.1% (91.7%, 74.4% and 56.7% for the respective risk groups). Allogeneic stem cell transplantation (alloSCT) was performed for 40% of patients in CR1. Median OS for all patients was 69 months (10 mo for patients in adverse risk group vs not reached in other risk groups, P<0.001). Among the cytogenetically normal AML patients (n=38), OS was superior in patients with NPM1 mutation, and shorter in patients with FLT3-ITD compared with others (25 mo vs not reached, p=0.0735). Moreover, within the adverse risk group the median OS was shorter in patients with monosomal karyotype than the others (7 mo vs 15 mo, P=0.034). Despite of low transplant-related mortality (13%), only patients in the adverse risk group tended to have an OS benefit from alloSCT over chemotherapy (16 mo vs 7 mo, P=0.14).
Conclusion: The risk stratification by the European LeukemiaNet effectively discriminated patients with AML into different outcomes in this single centre analysis. Treatment results are satisfactory only for favourable risk patients, and prognosis for patients with monosomal karyotype is particularly poor. The benefit of alloSCT was evident only for adverse risk patients.

P941

Biochip array analysis of various cytokines in patients treated for acute leukaemia

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Objectives: Cytokines have been studied as markers of immune system activation in various diseases including hematological malignancies. The objective of our study was to analyze plasma levels of various cytokines and growth factors by biochip array technology in acute myeloid leukemia (AML) patients.
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 cell transplantation were studied. We evaluated plasma levels of the following cytokines: interleukin-1 α (IL-1 α), interleukin-1 β (IL-1 β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), epidermal growth factor (EGF), monocyte chemotactic protein-1 (MCP-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 cytokines levels in active leukemia and in durable CR, we found significant decrease in plasma IL-1 β (2.56 ± 3.27 ng/L vs 1.63 ± 2.17 ng/L; $p < 0.05$), IL-6 (46.24 ± 83.14 ng/L vs 2.49 ± 2.51 ng/L; $p < 0.05$), IL-8 (104.99 ± 167.30 ng/L vs 11.72 ± 4.34 ng/L; $p < 0.05$), IL-10 (7.58 ± 14.15 ng/L vs 2.22 ± 4.78 ng/L; $p < 0.05$) and TNF-α (4.65 ± 4.27 ng/L vs 2.19 ± 1.13 ng/L; $p < 0.05$). On the other hand, we found significant increase in VEGF (63.93 ± 67.85 ng/L vs 114.39 ± 54.90 ng/L; $p < 0.01$) and EGF (16.48 ± 33.50 ng/L vs 64.42 ± 35.33 ng/L; $p < 0.001$). Plasma levels of other cytokines were without significant differences.
Conclusion: Our results indicate that plasma levels of some cytokines and growth factors (EGF, VEGF, IL-1 β, IL-6, IL-10, TNF-α) could serve as useful diagnostic and prognostic parameters for AML patients, showing activity of the disease. Further studies in a larger number of acute leukemia patients and comparing cytokine levels with healthy subjects will be needed to define the potential role of these and additional biomarkers in this context.
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P942

Comparable outcome of unrelated donor and related donor haematopoietic stem cell transplantation for intermediate-risk acute myeloid leukaemia – A single-centre analysis

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Background: allogeneic stem cell transplantation (alloSCT) using related donors improves prognosis of patients (pts) with intermediate-risk acute myeloid leukemia (IR-AML) in first complete remission (CR). The role of unrelated alloSCT for IR-AML in 1.CR remains to be evaluated. Recent advancement in HLA typing and supportive care can improve historically reported worse results of unrelated alloSCT. To evaluate the potential role of unrelated alloSCT in pts with IR-AML in 1.CR we retrospectively analysed the outcome of such pts transplanted in recent 5 years at our centre and we compared their outcome with a control group of pts transplanted for IR-AML in 1.CR with related donors.
Patients and methods: since 9/2005 37 consecutive pts (Group 1) with median of age 55 years (28-65 years) with IR-AML in 1.CR underwent unrelated alloSCT (70% matched, 30% mismatched). The control group (Group 2) of 25 pts transplanted with matched related donor did not differ for any significant prognostic variables (age, type of conditioning regimen, GVHD prophylaxis, etc) except for younger age of donors (p=0.0001) and higher amount of infused CD34+ cells (p=0.01) in the unrelated group.
Results: Group 1: all patients engrafted. Acute GVHD and chronic GVHD incidence were 30% (grade III-IV 5%) and 48%. With median follow-up 20 months (2-60 months) 26 pts (70%) are alive in 1.CR. 5 pts (14%) relapsed and died. 6 pts (16%) died due to NRM and one (3%) of them till day +100. The estimated probabilities of 3-years EFS and OS are 62% and 68%. Group 2: all patients engrafted. Acute GVHD and chronic GVHD incidence were 48% (grade III-IV 8%) and 48%. With median follow-up 33 months (10-60 months) 18 pts (72%) are alive (12 pts in 1.CR, 2 pts in 1.relapse). 4 pts (16%) relapsed (2 of them died). 5 pts (20%) died due to NRM and none of them till day +100. The estimated probabilities of 3-years EFS

and OS are 64% and 68%. We observed no significant difference in aGvHD (p=0,18), chGvHD (p=0,60), NRM (0,74), relapses (p=1.00), 3-years EFS (p=0.84) and OS (p=0,61) between both groups.
Conclusion: our data suggest that outcome of alloSCT for IR-AML in 1.CR was comparable between unrelated and related donors. Unrelated alloSCT due to low-risk of relapse and low-risk of NRM in recent years could be alternative treatment strategy for pts with IR-AML in 1.CR without related donor. But the final role of unrelated alloSCT for pts with IR-AML in 1.CR should be assessed in prospective trials.

P943
HLA-factors are associated with childhood ALL
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Objectives: ALL blasts routinely contain somatically acquired genetic abnormalities that provide insight into pathogenesis and strongly influence prognosis. Genetic and environmental factors play an interactive role in the development of childhood acute lymphoblastic leukemia (ALL). The HLA association has been considered as a possible genetic risk factor.
Aims: It is unknown whether there exists a restriction to certain MHC genotypes in leukemia like ALL.
Methods: The material of the study - leukocytes peripheral blood from 187 children with initial diagnosed ALL (B-ALL-98, T-ALL-37, ALL-52) and 2650 controls (leukocytes umbilical cord blood from healthy donors). Revealing HLA antigens at low level performed by SSO method on DynalRELI 48 processor. The results received with ambiguous interpretation was using PCR-SSP method (Ivitrogen).
Results: The HLA-factors of predisposition in childhood ALL changes in dependence on sex and immunocytologic type of the disease. The common HLA-factors of predisposition to B-ALL in childhood are groups alleles: HLA-C*04 (OR=1,9 p=0,007), DRB1*14 (OR=3.1 p =0.002) and DRB1*09 (OR=3.5 p=0.001); to T-ALL: HLA-DRB1*01 (OR=2.2 p=0.0035), DRB1*13 (OR=2.35 p=0.002) and DQB1*05 (OR=2.45 p=0.002). Characteristic markers of predisposition to development in boys with ALL are groups alleles: HLA-B*50 (OR=3.3 p=0.03), Cw*16 (OR=3.7 p=0.016), DRB1*14 (OR=5.1 p=0.0015); in girls - HLA-A*32 (OR=2.9 p=0.03), B*15 (OR= 2.6 p=0.02), delta*47 (OR=10.9 p=0.009) DRB1*03 (OR=2.2 p=0.04). A characteristic marker of predisposition to development in boys with T-ALL are groups alleles: HLA-A*26 (OR=3.7 p=0.006) and DRB1*13 (OR=2.55 p=0.03). A characteristic marker of stability to development in boys with ALL is groups alleles HLA-DRB1*03 (OR=0.3 p =0.037).
Conclusion: Thus, B-ALL and p-ALL have different HLA factors of predisposition, stability and force of association with disease depending on a sex of the child.

P944
HLA-factors are associated with childhood AML
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Objectives: Genetic and environmental factors play an interactive role in the development of childhood acute mieloid leukemia (AML). The HLA association has been considered as a possible genetic risk factor and heterogeneous disease.
Aims: It is unknown whether there exists a restriction to certain MHC genotypes in leukemia like AML.
Methods: The material of the study - leukocytes peripheral blood from 132 children with initial diagnosed AML (M2-AML-28, M4-AML-26, M5-AML -28; M7-AML-18; AML-32) and 2650 controls (leukocytes umbilical cord blood from healthy donors). Revealing HLA antigens at low level performed by SSO method on DynalRELI 48 processor. The results received with ambiguous interpretation was using PCR-SSP method (Ivitrogen).
Results: The HLA-factors of predisposition in childhood AML changes in dependence on sex and immunocytologic type of the disease. The common HLA-factors of predisposition to AML in childhood are groups alleles: HLA-C*05 (OR=2.22 p =0.05) and C*07 (OR=2.12 p=0.0001). Marker of strong association with M2-AML and M7-AML and high risk of development of diseases on population a level is HLA-C*07 (EF=53.8 % and EF=60.2 % accordingly); a marker strong association with M5-AML and high risk of development of disease on population a level is HLA-DQB1*06 (EF=57.3%). Characteristic markers of predisposition to development in childhood with M2-AML are group alleles: HLA-A*24 (OR=2.5 p=0.046), delta*51 (OR=3.07 p=0.03) and C*07 (OR=3.6 p=0.015); to M4-AML: HLA-A*25 (OR=2.8 p=0.05), B*07 (OR=2.36 p=0.049), C*05 (OR=3.97 p=0.004), DRB1*12 (OR=4.26 p=0.0077) and DQB1*06 (OR=2.36 p=0.049); to M5-AML: HLA-A*68 (OR=3.08 p=0.05), C*05 (OR=3.73 p=0.02), DRB1*07 (OR=2.67 p=0.048) and DQB1*06 (OR=4.33 p=0.0045); to M7-AML: HLA-B*39 (OR=7.02 p=0.03), B*49 (OR=8.4 p=0.019), C*07 (OR=4.25 p=0.03) and DRB1*11 (OR=3.9 p=0.016). The common marker of stability to disease AML in children is specificity HLA-DRB1*07 (OR=0.59 p=0.001). A characteristic marker of stability to development M4-AML in children is HLA-DQB1*02 (OR=0.35 p=0.05).
Conclusion: Thus, different variants AML have different HLA markers of predisposition, stability and force of association with disease.

P945
The role of second allogeneic haematopoietic cell transplantation on relapse of haematological diseases
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The treatment options on relapse of hematological disease after Allo-HCT are depends on original diagnosis, the patient's clinical condition and donor status. General approaches include cessation of immunosuppressive treatment, use of chemotherapy and donor lymphocyte infusion alone or combined with chemotherapy. Another option is to apply second allo-HCT. In this retrospective study, we purposed to give the outcomes of second allo-HCTs in patients who relapsed after first allogeneic transplantation. Between June 1988-Dec 2009 679 patients with benign and malign hematological disease underwent allo-HCT. Of these, 17 patients (2.7%) due to relapse or refractory of original disease was performed second allo-HCT from their same donor (n=13) or an HLA identical other sibling or relative donors (n=4) between Oct 1995 to Dec 2009. The clinical features of the patients and their donors prior to 1st and 2nd allo-HCT were given in table 1. Most of the diagnoses were acute leukemia (70.6 %) or chronic myeloid leukemia (23.6%). Ninety-one percent of patients with acute leukemia had active disease prior to second allo-HCT. The use of reduced intensity conditioning regimen was more frequent in second transplantation compared the first one (17.6% vs 58.8%, p=0.01). The stem cell source was mostly peripheral blood (94.1%) in the second transplantation. No immunosuppressive agent was given in two patients with active disease prior to second transplantation. The results of second allo-HCT were summarized in table 2. Complete remission was achieved in 76.5% of the patients. Acute severe GvHD (GrII-IV) occurred in 9 patients (60%). We observed chronic GvHD in 70 % of the patients who survived more than 100 days after the second transplantation. The probabilities of three-year relapse free and overall survival were 17.6%±9.2% and 20.2%±10.2%, respectively. When we repeated the statistical analysis in acute leukemia patients, relapse free and overall survival was estimated as 16.7%±10.7% and 19.4%±12.2%, respectively. In conclusion, second allo-HCT should be reconsidered as salvage treatment in relapse after first allo-HCT. Transplant-related mortality and relapse of original disease have continued to be main problems

in de novo leukemic patients (n=11). Therefore, new modalities should be developed to reduce the leukemic burden prior to transplantation and to increase the graft versus leukemic activity using different conditioning regimens and immunosuppressive agents.

Table 1: The patients' and their donors' features prior to first and second allo-HCT

Variables	1st allo-HCT	2nd allo-HCT
Diagnosis and disease status prior to transplantation		
AML	7 (41.2%)	7 (41.2%)
1st CR/2nd CR/Active disease	*4/2/1*	*0/1/6*
ALL	4 (23.5%)	4 (23.5%)
1st CR/2nd CR/Active disease	*2/1/1*	*0/0/4*
CML	4 (23.5%)	4 (%23.5%)
Early CR/Late CR/ARBC	*2/1/1/0*	*0/1/2/1*
SAA	1 (5.9%)	1 (5.9%)
2nd AML from CMML (refractory)	1 (5.9%)	1 (5.9%)
Median time between diagnosis and transplantation (range)	7.7 months (2.8-65.1 months)	29.1 months (9.7-176.9 months)
Median time between 1st and 2nd transplantation (range)		22.3 months (2.8-169.3 months)
The intensity of conditioning regimen		
Myeloablative	14	7
Reduced intensity	3	10
Immunosuppressant		
Present/Absent	17/0	15/2
Stem Cell Source (PB/BM)	9/8	16/1
Donor type, n		
HLA identical sibling	15	15
HLA identical relative	1	1
Singeneic	1	1
Other sibling/relative donor		3/1

Abbreviations: AML: Acute Myeloid leukemia, ALL: Acute Lymphoblastic Leukemia, CML: Chronic Myeloid Leukemia, SAA: Severe Aplastic Anemia, CMLL: Chronic Myeloid Leukemia, CR: Complete remission, CP: Chronic phase, AP: Accelerated Phase, BC: Blastic Crisis, PB: Peripheral blood, BM: Bone Marrow

Table 2: The outcomes of second allo-HCT

Variables	2nd allo-HHN
Response to transplantation: CR/No response/Not evaluated, n	13/2/2
The reasons of death, n	12/17 (70.6%)
Relapse and progression	8/17 (47.1%)
Transplant-related mortality	4/17 (26.6%)
Acute GvHD (Absent/GrI/GII-IV),n (Evaluable patients: n=15)	4/2/9
Chronic GvHD,n Limited/Extensive (Evaluable patients: n=10)	7 (70%) 1/6
Median time to from the 2nd transplantation to relapse, (range)	10.7 months (1.9-37.1 months)
The probability of relapse free survival	
12- month	35.3% ±11.6%
36- month	17.6%±9.2%
The probability of overall survival	
12- month	47.1% ± % 12.1%
36- month	20.2%±10.2%

P946

CD56 positivity is a poor prognostic factor in AML patients undergoing stem cell transplant

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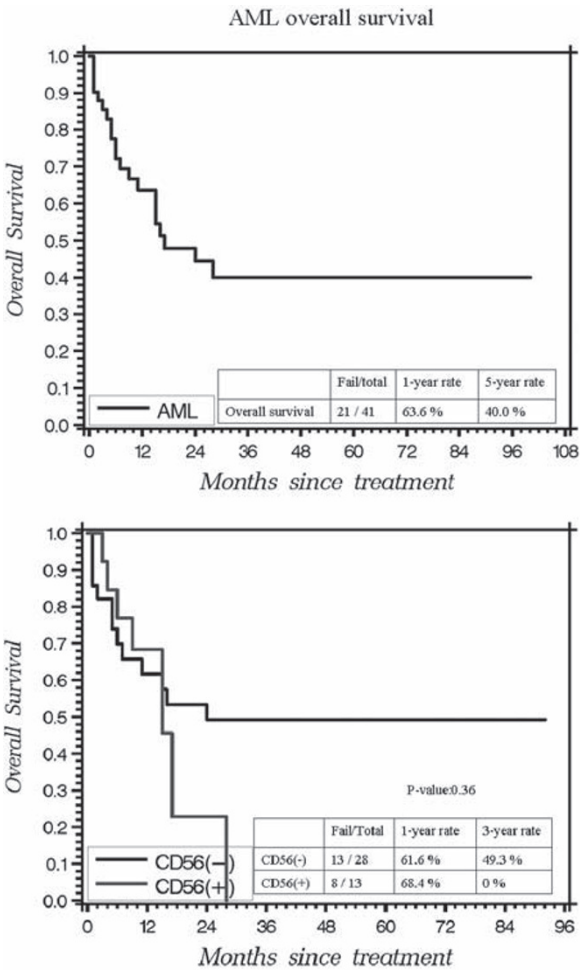
Background: CD56 positive AML patients have more extramedullary infiltrate and higher relapse rate and inferior outcomes. We evaluate the transplant outcomes in our CD56 positive as compared with CD56 negative AML patients.
Methods: Between August 2002 and September 2010, we have 41 AML patients undergoing haematopoietic stem cell transplantation at our institute. Two of them underwent autologous transplantation and 39 were allotransplant. We analyze the whole outcomes and relapse rates when transplanted in complete remission between CD56 positive and negative patients.
Results: In these 41 patients, 13 were CD56 positive (M/F 7/6 and median age 34.9 years) with 7 of them (53.8%) had extramedullary involvement; 28 were CD56 negative (M/F 14/14) and median age 42.7 years) with 2 of them (7.1%) had extramedullary involvement. Two-year and five-year overall survivals for all patients were 48% and 40%, respectively. For CD56 positive and negative patients, 2-year overall survivals were 22% and 54%, respectively. There were 30 patients undergoing transplant in complete remission with 8 CD56 positive and 22 CD56 negative, and the relapse rates after transplant were 75.0% and 31.8%, respectively, (p=0.049). High risk cytogenetics were found in 5 out of 13 CD56 positive patients and 5 out of 17 available cytogenetics CD56 negative patients.
Conclusion: Even under the most intensive treatment haematopoietic stem cell transplantation, CD56 positive AML patients still have more extramedullary involvement, more relapse rate, and poor survival even without significantly different high risk cytogenetics in either arms.



P947

Fludarabine with i.v. busulfan as novel conditioning regimen therapy for autologous stem cell transplantation in patients with acute non-lymphoid leukaemia: a single-centre experience

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Introduction: The gold standard for the treatment of Acute Non Lymphoid Leukemia (ANLL) after conventional chemotherapy remains the allogeneic transplant. Unfortunately, there is a group of patients that may be benefit from this type of transplant but is not feasible for various reasons, such as the absence of an HLA-matched donor, the presence of comorbidities, the advanced age. For these patients, autologous transplantation (ASCT) remains a viable alternative.
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 October 2010, we utilized a conditioning regimen with Flu (120 mg/sm) and myeloablative dose i.v. BU (12.8 mg/bw) (BUFlu) in 16 patients (7 females and 9 males; median age: 45.5 years, range: 6-59) with ANLL who received ASCT. At time of transplant, disease status was: 14 patients in first complete remission (CR), 2 in second CR. Patients were classified at high risk (31.5% of evaluable patients) when the white blood cell count at diagnosis was higher that 30×10^9 /L and/or cytogenetic 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 4.15x10E6/Kg (range 2.0–7.4) were infused.
Results: The 43% and 56% of patients did not need any transfusion therapy in terms of packed cells and platelets, respectively. Major complications observed were late mucositis (31% WHO 3-4) and fever (81% WHO 2). All patients engrafted. The overall median time for absolute neutrophil count (>0.5x10E9/l) was 16 days (r.12-20) and for platelet count (>20x10E9/l) was 14 days (r.12-22). The median duration of hospitalization was 26 days (r.23-34). At median follow-up of 14.5 months (r. 1-29), TRM was 0%. The OS, DFS and RR was 81.2%, 68.8% and 31.2% respectively. All relapsed patients belonged to high-risk group. Three patients died for disease recurrence.
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.

P948
Role of donor lymphocyte infusions following allogeneic stem cell transplantation in patients with acute lymphoblastic leukaemia
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Objectives: To evaluate efficacy of donor lymphocyte infusion (DLI) after allogeneic stem cell transplantation (allo-HSCT) in pediatric patients (pts) and young adults with acute lymphoblastic leukemia (ALL).
Patients and methods: Data from 22 pts with ALL receiving allo-HSCT from HLA-matched related donors (MRD) (n=8), matched unrelated donors (MUD) (n=8) and haploidentical donors (haplo) (n=6) was retrospectively analyzed. At the moment of allo-HSCT 10 pts had complete remission (CR), 6 pts were in relapse and 6 patients had resistant leukemia. 12 patients received myeloablative conditioning (MAC), 10 patients received reduced intensity conditioning (RIC).
After allo-HSCT 17 pts received DLI for disease relapse, 14 of them received prior cytoreductive chemotherapy. Minimal residual disease was indication in 5 pts. A total of 45 DLI was performed using an escalated dose regimen with initial dose of $1x10^4$ CD3+cells/kg. Nine pts received only one DLI with total cell dose (TCD) ranging from $1x10^4$ to $2x10^7$ CD3+cells/kg due to disease progression or graft-versus-host disease (GVHD). Patients responding to therapy (n=12) received 2-6 DLI (mean 2) with TCD ranging from $1.5x10^5$ to $1,2x10^8$ CD3+cells/kg (median $9x10^6$ CD3+cells/kg). In 12 pts TCD exceeded $5x10^6$ CD3+cells/kg. At the moment of DLI no pts had signs of acute GvHD, 5 pts had signs of chronic GvHD.
Results: Complete remission (CR) after DLI was obtained in 7 pts (32%): 4(33%) with MAC, 3(30%) with RIC. Three pts relapsed after DLI therapy. Four pts after allo-HSCT and DLI are alive and in CR. Response rate was similar in pts with MUD 3(38%) and MRD 3(38%), in the pts with haplo it was 1(17%). GvHD grade I-II developed in 5 (22%) pts. 1 patient developed grade IV GVHD after the first DLI. The 2yr OS was 30%, 13% in RIC, 42% in MAC group (p=0,19). The 1yr OS after DLI was 50% in pts after MRD and haplo HSCT, 63% in pts with MUD (p=0,9). The 1 yr OS was 67% in pts who received TCD exceeded $5x10^6$ CD3+cells/kg, 38% in pts received TCD less then $5x10^6$ CD3+cells/kg (p=0,01). 3 yr DFS was 40% for all pts. Duration of CR after DLI ranged from 1 to 38 months.
Conclusions: DLI is a promising therapy option for pts with post allo-SCT relapse of ALL. TCD exceeding 5x106 CD3+cells/kg, and additional cytoreductive chemotherapy may improve results.

P949
Effect of donor lymphocyte infusion in aplasia after reinduction chemotherapy for leukaemia relapse after allo-SCT
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Donor lymphocyte infusions (DLI) in patient with leukemia relapse after allogeneic stem cell transplantation (allo-SCT) are accept now as a way to augment the graft versus leukaemia effect. In the present study we performed a retrospective analysis of 16 adult patients with leukaemia (12 – AML, 2 – ALL, 2 – CML) who had received DLI due to hematological relapse after HLA-matched allo-SCT. The goal of our study was to investigate the effect of DLI during myelosupressive aplasia after reinduction chemotherapy.
Methods: Donor lymphocytes were infused once a week (9 pts) or with 2-3 weeks intervals (7 pts). Number of infusions was 2-4. Total lymphocytes dose varied from $3,1\ x10^8$ to $7,9\ x10^8$ cells/kg, and CD3+cells – from $0,9\ x10^8$ to $3,6\ x10^8$ cells/kg. DLI were applied on day 5-7 after completing reinduction chemotherapy in cytopenic phase. IL-2 (2 – 9 MUE) was used after every DLI. Chimerism was detected and monitored by PCR analysis (VNTR and STR) and by FISH-analysis for centromers of X and Y chromosomes.
Results: Among 16 patients treated with DLI+IL-2 in aplasia complete remission with complete donor chimerism was achieved in 12 (75%) pts (9 – AML, 1 – ALL, 2 – CML). 6 of them are alive for 2, 3, 3, 23, 46, 90 months after DLI; 2 pts died at 4 and 5 months after DLI due to GVHD-related infections; in 4 pts disease free survival was 2, 5, 5, 13 months followed by 2-nd relapse and in 2 cases extramedullary relapse was registered before hematological. Other 4 pts (3 – AML, 1 – ALL) did not respond to this DLI schedule. Incidence of acute GVHD was seen in 8 pts (4 – grade II and 4 – grade III-IV), 5 of them had later chronical GVHD.
Conclusion: Our results suggest, that a new approach to infuse donor lymphocytes in aplasia after reinduction chemotherapy is effective in relapsed after allo-SCT leukemia pts and needs further investigation.

P950
Post-remission treatment of acute myeloid leukaemia: chemotherapy versus haematopoietic stem cell transplantation, a Brazilian single-centre experience
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Aims: to evaluate the post remission treatment of AML with CT or HSCT (allogeneic or autologous).
Patients and methods: patients with AML diagnosis between January 1999 and December 2008 that achieved first complete remission (CR) were evaluated (acute promyelocytic leukemia was not included). Patients were treated with TAD-9 or 3+7 induction (Daunorubicin 60mg/m²) and 2 two courses of

consolidation with HD-ARAC (with 2g/m212/12h) and Daunorubicin (45 mg/m²). Patients with related HLA donor and good performance status were referred to HSCT after the first course of consolidation. Patients with no donor were submitted to peripheral blood stem cell collection after consolidation for autologous transplantation. If mobilization was not successful or feasible, patient was treated with consolidation CT. Disease-free survival (DFS) was calculated from CR date until death, last follow-up or relapse and overall survival (OS) from CR until death or last follow-up, using Kaplan-Meier method and log rank test.
Results: 73 AML patients were included, with median age of 39 years (11-60 years). Cytogenetic abnormalities were found in 18/57 patients (inv 16-5; trisomy 8-4; others-9). Median time to CR was 33 days (19-212 days). Fifty-nine patients (80%) achieved CR with one cycle of CT. After CR, 49 patients received one cycle of consolidation (67%) and 12 received two cycles (16%). Forty-four patients were submitted to HSCT (60%), 9 to autologous transplantation (12%) and 20 to CT (27%). Source of stem cells: bone marrow in 27 patients (51%) and peripheral blood in 26 (49%).
Conditioning regimen: Bu+Cy (44 patients) and CT + TBI (9 patients). Twenty-four patients relapsed (33%). There were 42 deaths (57.5%) due to: relapse (17-40%), infection– 19 (45%), GVHD (2-4.8%) and others (4). OS was 39% in a median time of 22 months (1-136) and DFS was 33% in a median time of 15 months (1-127). DFS was 40% in patients treated with transplant and 15% with CT (p= 0.004). OS was 45% transplant and 23% CT (p= 0.02) in 10 years.
Conclusions: there was a superior response in patients treated with transplant compared to CT. These results may be related to dose intensification and GVL effect and also to better overall support treatment in the transplant setting. Most deaths were related to disease and toxicity. In the future, incorporation of prognostic markers to guide therapy, use of higher doses of anthracyclines and better support may improve the AML patient prognosis.

P951
Safety and efficacy of allogeneic haematopoietic stem cell transplantation with myeloablative regimen in children with acute myeloid leukaemia: a single-sentre experience
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Introduction: Allo-HSCT in children with AML remains nowadays controversial issue. Even high risk patients not always are considered as a candidates for HSCT in first remission.
Aim of the study: In our retrospective study we analyzed the safety and efficacy of allo-hematopoietic stem cell transplantation with myeloablative regimen in children with AML in one institution.
Patients and methods: In our department between October 1999 and November 2010 a total number of 30 HSCT procedure was performed in 27 patients with high risk AML. 3 childrens were two times transplanted due to relapse after 1 st HSCT procedure. In our group were 14 girls and 13 boys, and median age was 9 yrs (range 7 months-18 yrs). At HSCT 14 out of 27 patients were in first complete remission (CR), 12 – in second CR, 1- in 3 CR and 2 children wasn't in CR (partial remission) of the disease.
Almost in all procedures (26/30) we carried out myeloablative conditioning. Chemotherapy was administered in 14 patients in 1st remission: Bu/Cy in 9 children, Treo/Cy in 3, Treo/Mel in 2 pts, and 1 girl received TBI/VP16 in 1st CR. 2 girls received non- myeloablative conditioning regimen to first transplant. 11 patients in advanced AML received melphalan in addition to Bu/Cy or Treo/Cy and in 3 procedures myeloablative regimen was TBI based.
In 30 procedures stem cell donor was HLA match (sibling in 14 and unrelated in 11, while in 5 cases we used haploidentical donor.
We also checked hematopoietic chimerism usingPCR or FISH method.
Results: All patients engraftment.
15 patients (56%) are alive and disease free and median observation time of surviving patients is 60 months (range 1-123 months). 11 of surviving patients were transplanted in 1 CR, 4 of surviving children were transplanted in 2 CR. Among the patients transplanted in 1st CR 3 patients (20%) died from transplant related complications (TRM): two children after myeloablative chemotherapy (multiorgan failure and infection)and one girl after non-myeloablative regimen (CNS toxicity). Three children transplanted in 2 CR died of GvHD . 7 patients (26%)relapsed after HSCT, 5 of them were re-transplanted from the same donor and two of them are alive.
Conclusion: In our opinion HSCT with myeloablative conditioning regimen is a reasonable clinical option for patients with high risk acute myeloblastic leukemia especially in first remission. In our cohort of patients surviving rate seemed to be higher as compared to standard chemotherapy alone.

P952
Autologous haematopoietic stem cell transplantation as an intensive consolidation therapy for adult patients in remisssion from acute myeloid leukaemia
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Despite advances in our understanding of its pathogenesis, acute myeloblastic leukemia remains difficult to treat. Although initial complete remission can be achieved in a high percentage of patients, relapse occurs in 70-80% of the patients. Two main approaches have been the attempt to eradicate the leukemic clonal cells population via chemotherapy with or without autologous stem cell rescue or to pursue a combined approach using an antileukemic therapy combined with an antileukemic immune response via allogeneic bone marrow transplantation. Autologous transplantation can be used as a consolidation therapy in the older population, and lack of a matched donor does not preclude the patients from this treatment.
We report a retrospective analysis on 55 patients diagnosed with de novo AML, who did not have an available histocompatible donor, and who underwent autologous transplantation between years 2000-2010 at the University hematology Clinic, Skopje, Macedonia. All patients had ECOG score 1 or less. The patient's age ranged from 17 to 65 years with the median age 41 years. There were 31 males and 24 females. For stem cell mobilization patients received chemotherapy or chemotherapy plus G-CSF. The preparative chemotherapy regimen prior to autologous transplantation consisted of BuCy in 31 patient, BEAM in 22 and BuCyMel was used in the remaining 2 patients. We used bone marrow as primary source of stem cells in 18 patients, and peripheral blood stem cells in remaining 37 patients.
The five years overall survival was 52% and the 5 years disease progression free survival were 42%. Factors that can influence the overall survival and the disease free survival such as: age, disease status, stem cell source, chemotherapy regiments prior to transplantation, conditioning regiments, number of mobilized stem cells, age and bone marrow stem cell source. We report that the clinical results of autologous hematopoietic stem cell transplantation are sufficiently encouraging to warrant future trials that include autologous transplantation as an option for appropriately selected patients with AML in CR1. We conclude that autologous hematopoietic stem cell transplantation is a reasonable and save intensive consolidation for patients with acute myeloblastic leukemia who do not have a suitable HLA –matched donor.

P953

Flag-IDA regimen as salvage chemotherapy before haematopoetic stem cell transplantation in the treatment of refractory/relapsed acute myeloblastic leukaemia: single-centre experience

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During the past several decades, improvements in chemotherapeutic regimens and supportive care have resulted in significant but modest progress in treating AML.Conventional chemotherapy is highly effective in the treatment of acute myeloblastic leukemia (AML). About 50-80% of adult patients with de novo acute myeloblastic leukemia achieve complete remission (CR) with currently available chemotherapy regimens consisting of antracyclines and cytarabine. However, relapse develops in more that 40% of the cases within two years, and 15-25% of patients fail to achieve complete remission because resistant to treatment or death. The management of cases with primary refractory and /or relapse disease is very difficult and prognosis in this subset of patients after several different chemotherapy combinations is still very poor with a CR rate 33-41%.

We evaluated efficacy and toxicity profiles of FLAG-Ida combination chemotherapy as salvage chemotherapy before hematopoietic stem cell transplantation in patients with refractory/relapsed AML.

At the University Hematology Clinic in Skopje, Macedonia, in the period 2006-2009, twenty patients with refractory/relapsed acute myeloblastic leukemia were treated with FLAG-Ida regiment. Patients were between 16-52 years old, 6 female and 14 male. They were treated with fludarabine 30 mg/m2, cytosine arabinoside (AraC) 2 g/m2 for 5 days, Idarubicin 10 mg/m2 for 3 days, and granulocyte colony stimulating factor G-CSF 5 mikrog/kg from day 0 till neutrofil recovery (ANC >1.0 x109/l). Complete remission were achieved in 9 patients (45%), four patients (25%) died of post chemotherapy complications, and 7 failed to achieve complete remission. Out of 9 patients who achieved complete remission, 4 went autologous bone marrow transplantation, 4 went allogeneic bone marrow transplantation, and 1 is being evaluated for the same. Major complication encountered were mucosistis, transient hepatic toxicity, fungal and bacterial infections.

Our experience confirmed that FLAG-IDA regimen is well tolerated and effective therapy in relapsed/refractory acute myeloid leukemia. FLAG-Ida is a good choice in cases with refractory/relapsed acute myeloblastic leukemia for salvage chemotherapy and it is wise to consolidate it with hematopoietic stem cell transplantation. Those patients included in the hematopoietic progenitor transplant program, clearly benefit from allogeneic or autologous BMT, obtaining a longer disease free survival and overall survival.

P954

Autologous stem cell transplantation in patients with acute myeloid leukaemia: results after long follow-up

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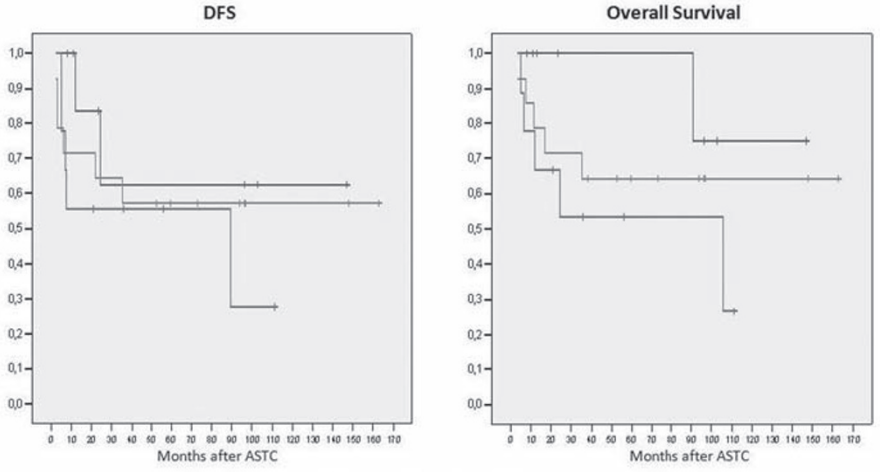
Autologous stem cell transplantation (ASCT) remain as a good procedure for postremission therapy in a subgroup of patients (pts) not candidates for allogeneic stem cell transplantation (SCT) with acute myeloid leukemia (AML) who obtain remission after chemotherapy. In this observational retrospective study we analyze, based on cytogenetic risk group, the results in pts with AML in CR1/CR2 who underwent an ASCT in our institution.

[P954]

Table 1. Characteristics of patients undergoing ASCT

N	31
Median age	45 (22-67)
Gender (F/M)	19/12
Risk category	
Good	8 = 1 t(8;21), 3 inv16, 1 t(15;17), 3 NC/NPM1+/FLT3-
Intermediate	14 = 10 NC*, 4 OTHER
High	9 = 5 secondary AML, 2 MLL, 2 OTHER
Disease status	
CR1	26
CR2	4

NC: normal cytogenetics, * molecular markers not available



Risk group	N	Follow-up, Mdn (range), months	DFS	OS
Good	8	23,5 (8-147)	83%	100%
Intermediate	14	93,5 (38,5-163)	57%	64%
High	9	46 (21-111)	56%	53%

Table 2. DFS, OS and median follow-up of patients in different risk groups

Patients: Between 1996 and 2010, 31 pts not candidates for allogeneic SCT underwent ASCT (Table 1) with a median age of 45 years (range 22-67). Eight showed good cytogenetic risk (25%), 14 intermediate (45%), and 9 high risk (29%). 26 were de novo AML and 5 secondary to hematological disease or chemotherapy. All of them were in CR at the time of transplantation: 26 in CR1 and 4 in CR2. Conditioning regimen was busulphan and cyclophosphamide in 25 and busulphan and fludarabine in 6. All received PB stem cells except for 3 pts who received BM+PB grafts.
Results: Median overall follow-up was 66 months (7.9-162). Median overall survival (OS) and median disease-free survival (DFS) for the different risk groups are shown in the figure and table 2. Although no statistical difference are seen due to number of pts in each group, a trend towards better DFS and OS are seen in better risk group categories. Twelve pts relapsed (38%) after ASCT in a median time of 6 months, 75% relapsed within the first year. Nine of them died in spite of allogeneic SCT performed in two, and 2 were rescued with 5-azacidine. An additional pt died due to second neoplasia. Transplant related mortality (TRM) at +100 days was 0%. Two patients showed secondary neoplasias (1 MDS and 1 breast cancer).
After a median follow-up of 66 months, 20 pts (64%) are alive, 18 of them in CR and without second neoplasias.
Conclusions: Limitations of this study include the number of pts included in each risk group and the availability of new molecular markers for risk categorization. However, our data show that ASCT offers a safe alternative as post-remission therapy for AML pts not candidates for allogeneic SCT, especially for those belonging to good and intermediate risk categories, providing a long-term DFS, with a 0% TRM and a low rate of second neoplasias. The new molecular markers should be used to define subgroups of patients who benefit most by this strategy.

P955
Defibrotide therapy in transplant-associated thrombotic microangiopathy is associated with improved overall survival compared to therapeutic plasma exchange
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Transplant-associated thrombotic microangiopathy (TA-TMA) has gained recognition as an important complication of allogeneic haematopoietic stem cell transplant (HSCT) and remains a highly challenging clinical problem. There is much controversy and uncertainties regarding the pathophysiology, diagnostic criteria and management of this disease. This retrospective study from 2004 to 2010 included 10 patients (4 males and 6 females) who underwent allogeneic HSCT at a median age of 37 years (range 16–67) and developed TA-TMA. They received treatment with defibrotide (DF), therapeutic plasma exchange (TPE) or both DF and TPE. TA-TMA was diagnosed according to the consensus criteria of the International Working Group of the European Group for Blood and Marrow Transplantation. The characteristics of our patients with the conditioning regimes, calcineurin inhibitor use for graft versus host disease (GVHD) prophylaxis and complications of infections and GVHD were analyzed. 7 patients had severe TA-TMA while 3 patients had mild TA-TMA. TA-TMA was diagnosed at a median of 57.5 days (range 25–322) after allogeneic HSCT and had a median duration of 32.5 days (range 5 -308). Two patients received treatment with DF, 5 patients had treatment with TPE and 3 patients had treatment with both DF and TPE. DF therapy was started at a median of 63.6 days (range 0–241) after the diagnosis of TA-TMA and was given intravenously at an average dose of 80mg/kg daily (range 400 mg–1200mg). Treatment was stopped after a median of 1 month (range 1–2). The median overall survival (OS) rates of patients who received treatment with DF compared to those who did not was not statistically significant with a p value of 0.115 ($p<0.05$). However, when the remission rates were compared between the same 2 groups, it was statistically significant with a p value of 0.0238 ($p<0.05$). In our study, it appears that patients treated with DF had a better survival rate (40%) compared to patients treated with TPE (10%) alone. However when the OS rate was analyzed, this was not statistically significant, though this could be due to our very small patient cohort. Of the 5 patients treated with DF, all achieved remission and showed stabilization of clinical and laboratory signs within 2 months of therapy. DF with its antithrombotic effect and low toxicity may be an effective strategy for TA-TMA but this requires confirmation with larger prospective studies.

P956
Allogeneic stem cell transplantation after conditioning FLU-BU12 (± ATG) in patients with acute myeloid leukaemia
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Objective: An outcome of patients suffering from AML allografting after conditioning FLU-BU12 (± ATG) combining fludarabine (total dose 150 mg/m^2), busulfan (total dose 12 mg/kg orally) with or without thymoglobulin (total dose 6 mg/kg) was retrospectively evaluated.
Patients and methods: 14 patients with AML (9 in complete remission, 5 with active disease) and the median age of 50 years (range 22-57) were allografted from sibling (3x), matched (6x) and mismatched unrelated (5x) donor after FLU-BU12 (± ATG) conditioning. Bone marrow and peripheral blood were used as a sources of stem cells in 3 and 11 recipients, respectively. Thymoglobulin was administered in 11 recipients. Graft versus host disease (GvHD) prophylaxis was realised with the administration of cyclosporine-A (CSP-A) only (5x), CSP-A and mycophenolate mofetil (MMF) (8x) or CSP-A and “short methotrexate (MTX) (1x). The median of posttransplant follow-up was 367 (range 91-1033) days at the time of outcome assessment.
Results: All patients engrafted. The numbers of neutrophils 0,5 x109/l and platelets 20x109/l were achieved in a median of 15 (range 11-45) and 11 (range 8-39) days after SCT, respectively. 100% donor chimerism was achieved in 10 patients (71%) on day +30. Non-hematological (gastrointestinal) toxicity grade III and IV was observed in 8 recipients (57%). 2 patients (14%) developed a mild form of liver venoocclusive disease (VOD). Acute GvHD of any grade was observed in 7 recipients (50%). Chronic form of GvHD was diagnosed in 2 of 10 (20%) assessable patients.
Overall non-relapse mortality (NRM) was 14% (2 patients). Posttransplant relapse or progression of AML was observed in 4 recipients (29%) and 3 ones (21%) died of disease progression. 8 patients (57%) lives in complete remission of AML. The probability of 3 years overall survival (OS) was 63%.
Conclusions: Regimen FLU-BU12 (± ATG) seems to be an alternative approach to the patients with AML and the need of significant antileukemic cytoreduction but standard myeloablative conditionings would be associated with the high risk of severe posttransplant complications and mortality.
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P957
Monosomy 7 and unrelated cord blood transplantation
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Monosomy or deletion of chromosome 7 (-7/del 7q) confers a poor prognosis to acute myeloid leukemia (AML) in adults. A recent study identified a weak graft versus leukemia (GVL) effect after standard allogeneic transplantation, specifically in patients associating – 7/del 7q and complex karyotype (1).

In contrast, we report 4 cases of -7 AML treated by cyclophosphamide fludarabine total body irradiation 2 Gy(TCF) reduced intensity conditioning followed by unrelated cord blood transplantation. Two of them had received a previous standard HSCT.
1-Female, 61 yo, date of diagnosis (dx) : 20 10 2006, M1, 45, -7, XX, one induction course, consolidation, related peripheral blood stem cell (PBSC) transplantation from her sister after fludarabine, TBI 2 Gy on 11 01 2007. Relapse 12 06 2008. Induction with high dose AraC and idarubicine, consolidation high dose AraC. Second transplantation with one cord blood unit after TCF on 28 11 2008. Date of last news: 17 11 2010, alive and well in second complete remission (CR2).
2-Male, 23 yo, dx: 22 02 2008, AML M0; 44,-7,der8, t(8;11)(p21;q23), der12, t(12;14)(p13;q11), -14, XY, absence of flt3ITD, presence of MLL rearrangement, one course of induction, consolidation, unrelated PBSC transplantation after busulfan cyclophosphamide on 02 07 2008. Relapse 17 03 2009. Induction with MIDAM and consolidation with same protocol. Second transplantation with one cord blood unit after TCF on 14 08 2009. Date of last news: 11 10 2010, alive and well in CR2.
3-Female, 66 yo, dx: 04 09 2008, AML with myelofibrosis, 45, -7, XX, absence of flt3ITD or MLL rearrangement, complete remission after 2 courses of chemotherapy, consolidation, unrelated cord blood transplantation after TCF on 13 03 2009. Early relapse post transplantation: 12 08 2009. Death of AML on 15 10 2009.
4-Female 65 yo, dx : M0, karyotype: 45, -7, XX, absence of flt3ITD or MLL rearrangement, one course of daunorubicine AraC and consolidation; unrelated cord blood transplantation after TCF on 30 04 2009. Date of last news: 06 12 2010, alive and well in first CR.
The duration of second CR, longest than the duration of the first CR, could suggest a specific GVL effect of cord blood against -7 AML. The follow-up remained short for all patients, however these results supported a possible way to treat AML with monosomy 7.
(1) A. Buzyn et al. Poor outcome after allogeneic HSCT for de novo AML with chromosome 7 abnormalities: a report for the ALWP of the EBMT. Blood 2007.

P958

Fludarabine, cytarabine and attenuated-dose idarubicin (m-FLAI) induction for elderly patients with acute myeloid leukaemia: result of a multicentre phase II study

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Background: We planned to evaluate the efficacy and safety of modified FLAI (m-FLAI) regimen in elderly patients with acute myeloid leukemia (AML).

Methods: Elderly (≥ 60 years) AML patients with adequate organ function who did not receive chemotherapy previously were included in this phase II study. We planned to enroll 107 patients to achieve primary end point of complete remission (CR) rate ≥ 60%. Patients received consecutive two cycles of m-FLAI chemotherapy as an induction. m-FLAI regimen was comprised of fludarabine (25 mg/m², day 1-4), cytarabine (1000mg/m², day 1-4) and attenuated dose idarubicin (5 mg/m², day 1-3).
Results: A total of 108 patients were enrolled in this study. Their median age was 68.4 years (range 60.3–81.2 years) and male to female ratio was 64:44. During study, 19 patients were dropped out from the study without treatment-related mortality (TRM). When analysis was performed in 89 patients, CR rate was 62.9% and TRM rate was 25.8%. Median overall survival (OS) of these patients was 9.3 months, and median event-free survival was 6.6 months. Complete bone marrow function recovery was achieved in 55.1% of patients after first m-FLAI induction, while in 71.2% of patients after second m-FLAI induction. Performance status, Charlson comorbidity index did not have either prognostic or predictive value in these patients. Risk group based on karyotype marginally influenced TRM rate (p=0.088) and OS (p=0.073).
Conclusions: Modified FLAI is a safe and very effective induction regimen for previously untreated elderly AML patients.

P959

Myeloperoxidase expression is a predictable prognostic factor in acute myeloid leukaemia: individualized treatment approach identifying patients to benefit from transplant

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Objective: Currently, the most common indication for allogeneic hematopoietic stem cell transplantation (alloHSCT) is Acute myeloid leukemia (AML). Despite extensive study, the use of alloHSCT in AML vary considerably. The decision of which of treatment options to choose is complex and depends on both clinical and molecular variables as well as the availability and histocompatability of donor stem cells. The importance of cytogenetics as an independent prognostic factor was clearly shown in a previous MRC report. but, there are several limitations in the use of karyotype as a risk stratification tool. Myeloperoxidase (MPO) is the hallmark enzyme of the myeloid lineage. A few studies have previously shown the prognostic significance of MPO in AML. However, so far there is no clear explanation on whether the expression of MPO relates to the prognosis of AML. We retrospectively analyzed the prognostic significance of the MPO expression in the 140 patients with diagnosed AML treated at a single institution.
Methods: Between January 2006 to August 2010, a retrospective analysis was on 140 patients with newly diagnosed AML at the division of hematology, the Yonsei university college of medicine. Patients were categorized into two groups according to MPO expression; 86 patients(61.4%) with MPO-positive (MPO+) and 54 (38.6%) with MPO-negative(MPO-). Of the 140 patients who diagnosed AML, 46(32.9%) had received transplants, including 32 patients who got alloHSCT from a matched sibling (n=21), unrelated (n=16) donor and 9 patients who got autoHSCT.
Results: By the univariate analysis, MPO was significant factors associated with OS and DFS. The OS at 1 year were 46.5% in MPO+ group and 24.1% in MPO- group (p=0.008). The DFS at 1 year was also different (34.9% and 16.7%, p=0.018). the DFS of the patients received transplants in the MPO+ group was equal with that in the MPO- group(p=0.221) while the DFS of the all patient in MPO+ group was superior than that in MPO- group. Although our study included a limited number of cases, transplant demonstrated an important role in improving clinical outcomes in AML. This results represented that negative prognostic effect of MPO- group was overcame by receiving transplants if suitable donor exist.
Conclusion: we suggest that MPO expression at diagnosis is a predictable prognostic factor in AML patient and It helps a individualized treatment approach potentially identifying those patients likely to benefit from transplant.

P960

Stem cell transplantation in acute promyelocytic leukaemia: the Iranian experience

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Introduction: Acute promyelocytic leukemia (APL) is a fairly rare form of acute myelogenous leukemia (AML) With M3 morphology and specific chromosomal translocation t(15;17) is

considered Stem cell transplantation (SCT) is a useful treatment for this malignancy.
Patients and methods: In this center, 20 Acute promyelocytic leukemia patients, (10 male and 10 female) with a median age of 25years (range: 3-46 years), received SCT. All of the patients were M3 morphology and specific chromosomal translocation t (15; 17).Between diagnosis and the start of the conditioning regimen time, 4 patients received only Arsenic trioxide(ATO), 5 patients received only all-trans-retinoic acid (ATRA) and 8 patients received ATRA+ATO. The disease status at the last evaluation prior to the conditioning was complete remission CR1 (6 patients), CR2 (6 patients), CR3+ (8 patients).Transplant types were divided into autologous (n=3), allogeneic transplants (n = 17). The sources of hematopoietic cells were 18 peripheral blood, 2 bone marrow, followed by infusion of autologous and allogeneic SCT. The donor types for the total of allogeneic SCT patients were human leukocyte antigen matched-identical siblings. Afterwards, the results were analyzed.
Results: The median time to an Absolute Neutrophil Count ≥0.5*10^9/L was +12. The median time to an Absolute platelet count ≥20*10^9/L among 18 patients was +16 and 2 patients never dropped below 20*10^9/L. Acute Graft versus Host Disease (GvHD) and Chronic GVHD occurred in 9(45%) and 6(30%) patients, respectively. The median follow-up time was 3.5 years (1.5 months-9 years). At present, 13(65.8%) are still alive. The main cause of death was relapse in 5 (71.4%) patients out of 7 died patients. The 4years, disease-free survival (DFS) was 56.1% and the 4years, overall survival (OS) was 63.1%.
Conclusion: The results of this study have shown that APL patients can achieve long term disease control by SCT.

Reduced-intensity conditioning

P961
Fludarabine and treosulfan conditioning for allogeneic stem cell transplantation; a dose- intense regimen with limited toxicity
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Allogeneic stem cell transplantation (SCT) is a potentially curative approach for patients (pts) with various hematologic malignancies. Standard myeloablative conditioning (MAC) is limited to younger, medically fit pts. Reduced-intensity conditioning (RIC) is feasible in pts not eligible for MAC, however relapse rates are increased, especially in pts not in remission at SCT. Novel approaches to deliver dose intense conditioning but with RIC type toxicity are in need. We explored a regimen consisting of fludarabine 150 mg/m² and tresulfan 30-36 gr/m² (FT) in 105 pts not eligible for MAC, median age 57 years (20-76) with. AML (n=56), MDS (n=33) or other diagnoses (n=16). The donor was HLA-matched sibling (n=50) or matched unrelated (MUD, n=55). Disease status was early (n=17), intermediate (n=24) or advanced (n=64) by standard criteria. 30 pts had comorbidity score ≥ 3 and 22 pts had a karnofsky score of ≤ 80 at of SCT. 27 pts had a prior SCT; 13 autologous and 14 allogeneic (12 from a different donor). 95 pts engrafted in a median of 12 days (7-23). With median follow-up of 16 months (1-73), 57 pts are alive and 48 died. The cumulative incidence of acute GVHD, acute GVHD grade III-IV and chronic GVHD was 26%, 11%, and 53%, respectively. The 3-year cumulative incidence of relapse and non-relapse mortality was 30% (95%CI, 21-44) and 25% (18-35), respectively. The median 3-year overall (OS) and progression-free survival rates were 45% (33-57) and 37% (26-49). Considering that only 23% of pts were in CR at SCT, these rates seem promising. Advanced disease and low Karnofsky score were adverse prognostic factors in multivariate analysis; hazrad ratios 3.7 (p=0.03) and 4.2 (p=0.001), respectively. Best results were obtained in pts with previously untreated MDS (n=33, including 28 pts with >10% marrow blasts at SCT) and pts with high-risk AML in first CR (n=14), with 3-year OS rates of 61% (42-80) and 65% (38-93), respectively. Interestingly a second SCT was not associated with a worse outcome. OS was 43%, 46% and 40% in first SCT, prior autologous and prior allogeneic SCT, respectively. In conclusion, FT is feasible in pts with advanced hematological malignancies not eligible for standard MAC. Promising results have been observed in pts with early stage disease, pts with advanced MDS and in pts failing a prior SCT. The relatively low relapse risk suggests that this regimen can be considered a dose-intensive regimen but with relatively favorable toxicity.

P962
The impact of anti-HLA antibodies on allogeneic haematopoietic stem cell transplantation after reduced-intensity conditioning regimen
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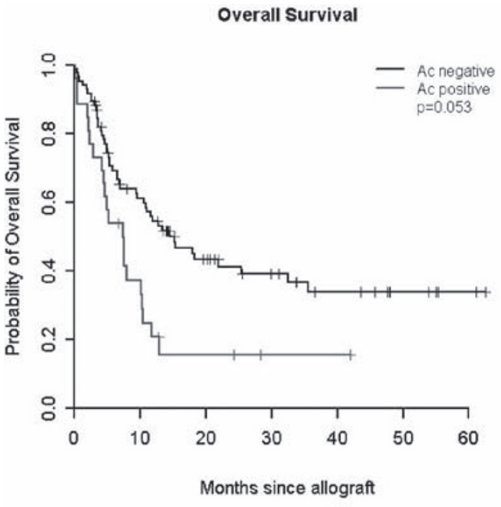
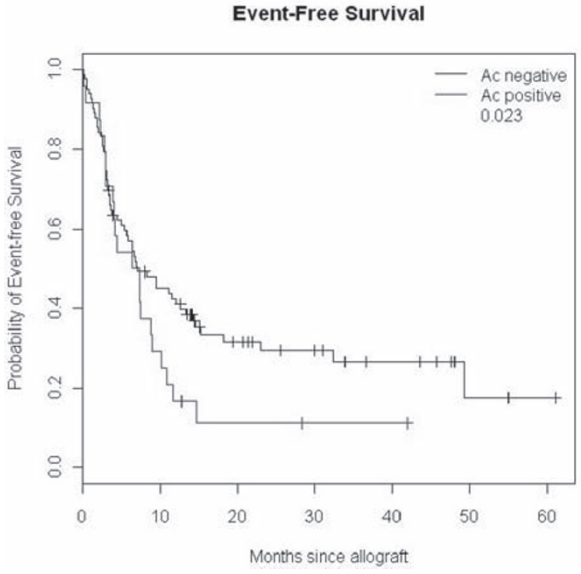
Background: Anti-HLA antibodies are associated with several complications in solid organ transplantations but their impact after allogeneic hematopoietic stem cell transplantation (HSCT) is not very well defined yet.
Patients and methods: To investigate the relevance of anti-HLA antibodies on allogeneic HSCT outcomes, we have retrospectively analyzed 107 peripheral blood allogeneic HSCT after reduced-intensity conditioning (RIC) regimen performed in our center between 2005 and 2010. RIC regimen consisted of total-body irradiation (200 cGy) and fludarabine (n=34, 32%), or busulfan and fludarabine (n=46, 43%) or FLAMSA regimen (n=27, 25%). Acute myeloid leukemia and myelodysplastic syndroms (n=64, 60%) were the most common diagnosis in the cohort.The detection of anti-HLA antibodies was performed before transplantation. Pre-transplant variables included age, gender, type of donor, disease and status of the disease before transplantation. Outcome variables included neutrophil recovery, incidence of graft-versus-host disease (GvHD), incidence of relapse, overall survival (OS) and event free survival (EFS).
Results: Twenty-four patients (22%) in the cohort had anti-HLA antibodies. The presence of anti-HLA antibodies was significantly correlated with patient gender. There were 54% of female positive patients versus 9% of male positive patients (P < 0.0001). Anti-HLA antibodies were also correlated with kind of donor (related vs unrelated, 15% vs 44%, p=0.002). There was neither association with other pretransplant variable nor with engraftment, incidence of relapse or acute GvHD. The presence of anti-HLA antibodies was associated with worse survival in univariate analysis (HR 2.04 [1.21-3.44], p=0.0056) and in multivariate analysis (HR 2.63 [1.32-5.25], p=0.006). The 3-year probability of OS was 34% (24-49) without anti-HLA antibodies and 16% (6-41) in their presence (Figure 1). Moreover anti HLA antibodies had a significant impact on EFS (HR 2.10 [1.1-4], p=0.023) with a 3-year probability of 26.5% (17.3-40.4) without anti-HLA antibodies versus 11.1% (3.4-36.9) in their presence (figure 2). In addition, anti-HLA positive patients had complications such as microangiopathy after transplantation with renal impairment (n=5, 20%) and cardiac injury (n=5, 20%).
Conclusion: In conclusion, our study supports that patients' pre-transplantation anti-HLA antibodies should be tested and considered as an important prognostic factor for OS and EFS after RIC HSCT.





P963

Treosulfan-based conditioning regimen for allo-SCT in 283 patients: co-morbidity index and disease status are predictors of outcomes

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Background: Allogeneic hematopoietic stem cell transplantation (allo-SCT) from an HLA-matched, related (MRD) or unrelated donor (MUD), is a curative option for patients (pts) with high-risk hematologic disease. In the absence of an MRD or MUD, pts have been offered investigational transplant strategies such as umbilical cord blood (UCB) or family haploidentical SCT (haplo-SCT). In an intention to treat analysis we, and others, recently showed comparable outcomes among the four major donor source. We are now reporting the analysis of the outcome after the stratification of patients receiving a treosulfan-based conditioning regimen according to two published prognostic indices: the hematopoietic cell transplantation comorbidity index (HCT-CI) and the Pretransplantation Assessment of Mortality (PAM) score.

Methods: The comorbidity and outcome analysis were determined retrospectively for consecutive patients who were transplanted at our Institution.

Results: Between January 2004 and November 2010, 394 pts received an allo-SCT in the fulfillment of the EBMT recommendations. Complete data for evaluation of HCT-CI were available for 283 pts (pts and disease characteristics are listed in Table 1): 244/283 pts were evaluable also for PAM score.

The 1y/3y overall survival (OS) for pts transplanted in complete remission (CR) is 72/56% respectively, for pts transplanted in progression of disease (PD) 33/24% (p<0.0001).

The 1y/3y overall OS according to age is comparable (57/46% <60y, 43/32% ≥ 60y) (p ns).

The overall 1y/3y OS according to HCT-CI stratification is 85/72% score 0, 56/42% score 1-2, 58/42 score 3-4, 31/23% score ≥5 (p<0.0001), according to PAM score is 100/75% (score ≤16), 80/63% (score 17-23), 50/38% (score 24-30), 39/20% (score >30) (p<0.0001). For pts transplanted in CR the 1y/3y OS according to HCT-CI stratification is: 94/74% score 0, 66/45% score 1-2, 76/57% score 3-4, 53/47% score ≥5 (p ns). For pts transplanted in PD the 1y and 3y OS according to

Table 1. Patient and disease characteristics

	283 patients
Age, years	
Median	47
Range	15-76
Over 60	59
Follow-up, months	
Median	20
Range	0.2-72
Conditioning regimens, "n" and %	
Treosulfan based	263 - 93%
Others	20 - 7%
Disease diagnosis, "n" and %	
Acute Myeloid Leukemia	147 - 52%
Acute Lymphoblastic Leukemia	43 - 15%
Myeldysplastic syndrome	24 - 8%
Non Hodgkin Lymphoma	27 - 10%
Hodgkin Disease	16 - 6%
Others	26 - 9%
Disease status at transplant, "n" and %	
Complete Remission (CR)	153 – 54%
Progression of Disease (PD)	130 – 46%
Donor Type, "n" and %	
MRD	60 – 21%
MUD	75 – 27%
UCB	12 – 4%
Haplo-SCT	136 – 48%
HCT-CI score distribution, "n" and %	
0	25 – 9%
1-2	61 – 21.5%
3-4	136 – 48%
>/=5	61 – 21.5%
PAM score distribution, "n" and %	
</=16	6 – 3%
17-23	69 – 28%
24-30	145 – 59%
>30	24 – 10%

Table 2. a. Univariate and **b.** Multivariate Analysis

2.a		**2yOS (%)**	**p**		
Age	< 60	46+/-4	**0.17**		
	>/= 60	40+/-7			
HCT-CI	0	75+/-10	**0.003**		
	>0	42+/-4			
Disease Status	CR	61+/-5	**<0.001**		
	PD	25+/-4			
2.b		**HR**	**95% IC**		**p**
HCT-CI Score	0	1.0			**0.012**
	>0	3.143	1.284	7.695	
Disease Status	CR	1.0			**0.0001**
	PD	2.791	1.976	3.942	

Higher HR was associated with poorer outcome

HCT-CI stratification is: 66/66% score 0, 36/36% score 1-2, 39/24 score 3-4, 15/5% score ≥5 (p0.0012). The evaluation per donor type confirms the presence of a trend according to the HCT-CI score. The evaluation of OS in multivariate analysis, according to disease status at transplant and HCT-CI score, confirms the independent value of these variables (Table 2).
Conclusion: HCT-CI score and disease status at transplant are confirmed to be useful predictors of outcome after a reduced-toxicity conditioning.

P964

First line non-myeloablative allogeneic stem cell transplantation in consolidation to autologous transplant in mantle-cell lymphoma: results of a pilot study

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Background: In 2002, we initiated a pilot study investigating the feasibility of non myeloablative (NMA) allogeneic stem cell transplant (SCT) in consolidation to autologous SCT as part of first line therapy for MCL.
Patients and Method: Newly diagnosed MCL patients referred to our center were offered participation in a tandem approach therapy. First line treatment consisted of 6 to 8 cycles of R-CHOP. Chemosensitive patients underwent an autologous SCT with BEAM conditioning. At day +100 post-SCT, consenting patients with an HLA identical sibling received out-patient non-myeloablative (NMA) SCT with fludarabine + cyclophosphamide conditioning and tacrolimus + MMF graft-versus-host disease (GVHD) prophylaxis. An unrelated donor transplant was proposed to patients younger then 50 without an identical sibling. Patients with chemo-refractory or recurrent disease were proposed second line salvage treatment. Chemosensitive patients underwent a tandem transplant while chemorefractory patients received a standard myeloablative SCT.
Results: A total of 33 patients referred to our center with MCL received a SCT. 21 received an allogeneic SCT and 12 a single autologous SCT. Sixteen patients were evaluated for first line tandem approach while 17 for relapsed or resistant disease. 11/16 MCL patients, median age 53, underwent an allogeneic SCT as part of their first line treatment while 5/16 median age 57, were treated with an autologous SCT. In the first line allogeneic setting, the 100 days and 1 year TRM were respectively 0% and 18%. Four patients died at 8, 12, 24 and 48 months post allo-SCT from TRM (3/4) or recurrent disease (1/4). After a median follow-up (FU) of 40 months, estimated 4 years overall survival (OS) is 70%. Of the 17 patients referred for recurrent or refractory disease, 10/17 median age 52, received an allogeneic SCT. 6/10 received a NMA SCT while 4/10 received a myeloablative SCT. In the recurrent or refractory allogeneic setting there was no TRM during the first 100 days. Five patients died at 6, 7, 14, 15 and 16 months post transplant (4/5) from TRM and (1/4) relapse. After a median FU of 39 months, the 3 years OS is 50%. Relapse (50%) was the major cause of treatment failure after autologous SCT while TRM (28%) was the major cause of failure post allogeneic SCT.
Conclusion: Allogeneic SCT is associated to a low TRM and should become the modality of choice in chemosensitive disease. It offers a promising approach to first line treatment of MCL.

P965

A phase II study of Yttrium-90-ibritumomab tiuxetan in combination with a fludarabine-based reduced-intensity regimen followed by allogenic stem cell transplantation in patients with relapsed or chemorefractory CD 20 positive non-Hodgkin's lymphoma

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Background: Phase II study (Clinical Trials.gov, NCT 00607854) to valuate the safety and efficacy of Yttrium-90 ((90)-Y)-ibritumomab tiuxetan in combination with a fludarabine-based RIC followed by allo-SCT.
Patients and methods: Patients with relapsed or refractory CD 20 positive NHL with a sensitive disease (at least PR) to the last salvage regimen and a suitable donor:related (MRD) or unrelated donor (MUD or with 1 mismatch). Each patient received Rituximab (250 mg/m² on day -21 and -14), a single dose of ((90)-Y)-ibritumomab tiuxetan (0,4 mci/Kg on day -14) followed from day -6 by a combination of fludarabine (30 mg/m² d -6 to d -2), busilvex ((3,2 mg/Kg d -5 and d -4) and antithymocyte globulin (2,5 mg/Kg d -1). GVH prophylaxis was based on cyclosporine (CsA) alone or in combination with methotrexate (Mtx). The trial was designed to enroll 30 evaluable pts. The study started on January 2008 and the last patient was included on October 2011. The primary objective was TRM at day 100. Secondary objectives were response (CR, PR) and EFS at 1 year. We report the safety data and the preliminary results.
Results: Twenty nine pts are evaluable for the PO. Median age was 57 years (32-64), and sex ratio (M/F) 22/8. Prior disease was DLBCL (10), MCL (9), FL (9) and MZL (2). The median number of previous regimens was 3 (2-5) and 29 pts had undergone ASCT before. Median time between diagnosis and allo-SCT and between ASCT and allo-SCT was 36 mo (range 1.3-108) and 18 mo (range 2-57) respectively. At time of transplant 18 pts were in CR and 12 in PR. There were 20 MRD, 8 MUD transplants and 2 pts with one mismatch. All pts received PBSC transplantation and GVH prophylaxis with CsA alone (29) or CsA + Mtx (1).
Three pts died in CR from a-GVH (2pts) at day 40 and 117 post-transplant respectively and from multi organ failure (1 pt) at d70. One pt died at d114 from progression. The TRM at day 100 was 7%. The median time to ANC engraftment (ANC > 500/mm3) was 17.5 days (range 12-22) and time to platelets engraftment (Plt > 20.000/mm3) was 10 days (range 0-23). Grade > 2 aGVH occurred in 3 pts. With a median follow-up of 3,7 month (range 1–30), 26 pts are alive, 22 in CR and 4 in PR and the estimated event-free survival at 1 year is 82% (CI 75%-87%).
Conclusion: RIT is safe and well tolerated when used in combination with a fludarabine-based RIC regime. The TRM is not increased by adding RIT in this conditioning regimen and also preliminaries the results seem to be promising.

P966

Genetic variability at loci controlling glutathion homeostasis affects transplant-related mortality and overall survival in patients receiving an allogeneic HSCT after a busulfan-based conditioning regimen

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Busulfan is the most widely used drug for allogeneic conditioning regimens. Busulfan metabolism depends on liver glutathion (GSH) availability. A number of loci controls liver GSH synthesis and consumption, occurring during drug conjugation and oxidative stress response.
Here we report on a study population of 331 consecutive patients who received an allogeneic HSCT for haematological malignancies at Institute of Hematology "Seràgnoli" from 2004 onwards. 185 patients received busulfan in the conditioning regimen, mostly at myeloablative intensity, while 146 received a TBI based regimen or a non busulfan RIC; clinical variables were similarly distributed in the two groups.
The impact of polymorphisms at loci involved in GSH balancing on overall survival (OS) and transplant related mortality (TRM) was tested.
A total of 35 polymorphisms (32 SNPs and 3 insertion/deletions) at 15 candidate genes were analysed by high throughput mass array Sequenom TM platform or by DHPLC.
We found that a C to G rs2180314 SNP at Glutahione Transferase A2 (GSTA2) locus (Codon 112 which leads to a Ser to Thr aminoacidic transition) impacts OS and TRM in the whole population (CC vs G-carriers: HR=1.604, 95%CI=1.081-2.381, p=0.019 for OS and HR=1.992, 95%CI=1.100-3.609, p=0.023 for TRM).
Such an effect was particularly evident in patients who received busulfan (CC vs G-carriers: HR=2.438, 95%CI=1.446-4.108, p=0.0008 for OS and HR=4.580, 95%CI=2.005-10.461, p=0.0003 for TRM). No effect was present in the group not receiving busulfan.
The polymorphism at microsomial GST-1 promoter (rs7970208) also affects OS and TRM, although to a lesser extent (AA vs G-carriers: HR=1.405, 95%CI=1.076-1.835, p=0.012 for TRM and HR=1.255, 95%CI =1.050-1.499, p=0.012 for OS).
These data point out that genetic variability at loci controlling GSH balancing may affect allogeneic HSCT outcome in busulfan treated patients.

P967

Improved survival following reduced-intensity cord blood transplantation using GvHD prophylaxis of tacrolimus + mycophenolate mofetil compared to tacrolimus alone

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Background: Although the application of cord blood transplantation (CBT) has been extended to elderly patients, final outcome has not been satisfactory. One of the issues regarding patients in this age range is that they have been particularly vulnerable to immune-mediated complications which resulted in high non-relapse mortality (NRM) early after transplantation (BBMT 2008;14:583).
Patients and methods: Patients > 60 years old with hematological diseases who underwent CBT from Jan. 2000 to Aug. 2010 were retrospectively reviewed. Among 147 recipients in total, 33 patients were excluded due to poor performance status (ECOG PS score 4) or had active infections at transplant.
Results: Median age was 64 (range, 60-82), 83 male, 31 female, and diagnoses included were AML (n=79), ML (18), ALL (8), SAA (4), and CML (5), in which 77% were in high risk disease status (AML/ALL/ML not in remission, CML blastic phase, or MDS RAEB). Poor PS 2 (31) or 3 (5) were included. Pretransplant conditionings were all fludarabine-based regimen combined with busulfan, melphalan, or TBI. Sixty-three (55%) received tacrolimus+MMF (FK+MMF) as GVHD prophylaxis whereas 50 (44%) were either ciclosporin A or tacrolimus alone. Median total nucleated cells and CD34+ cells infused were 3.05 (2.24-4.5) x 107/kg and 0.85 (0.31-1.65) x 105/kg. Cumulative incidence of neutrophil recovery (>500/ul) up to 50 days post-transplant was 78% (median 20 days post-transplant, range, 11-53). Median observation period of survivors post-transplant was 395 (26-2057) days. Cumulative incidences of NRM and relapse at 1 year post-transplant were 43.8% and 32.3%, respectively. Overall survival (OS) and event free survival at 1 year were 35.2% and 22.9%, respectively. In univariate analysis, GVHD prophylaxis using FK+MMF showed reduced NRM rate at 1 year (31.4%) compared to the others (60.8%, P=0.001), while relapse rates were comparable (38.4% vs 24.1%, respectively, P=0.20). In multivariate analysis assessing factors affecting OS, GVHD prophylaxis (FK+MMF; 47.6% vs others; 19.8% at 1yr) and disease status at transplant (standard; 40.1% vs high; 33.6%) showed statistical significance (P=0.0007 and P=0.036, respectively).
Conclusion: For elderly patients > 60 years, GVHD prophylaxis using FK+MMF showed favorable survival by reducing NRM, most likely due to better control of early immune-mediated complications.

P968

Fludarabine, low-dose busulfan and antithymocyte globulin compared to fludarabine and low-dose TBI for reduced-intensity conditioning prior to allogeneic stem cell transplantation in patients with lymphoid malignancies

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The patients and diseases for which allo-SCT is now considered have increased considerably over the past years especially thanks to the introduction of the RIC regimens. However there is a wide variability in the degree of myeloablation between the different protocols, and the toxicity profile might vary from one to another. The combination of fludarabine and Busulfan (usually 8 mg/Kg total dose) with or without ATG, is among the most used RIC protocols.
In order to decrease the toxicity of the transplant procedure, we hypothesized that further reduction (50%) of the Busulfan dose can improve transplant outcome for lymphoid neoplasms. Thus, this retrospective analysis was performed to assess whether a RIC regimen including fludarabine (120 mg/m²), low dose busulfan (4 mg/Kg total dose) and ATG (Thymoglobuline®, 5 mg/Kg)(FB1A protocol, n=44) is a valid alternative to the classical RIC regimen including fludarabine (90 mg/m²) and TBI (2 Gy)(FTBI, n=27).
The cohort included 37 males (52%) and 34 females (48%) treated consecutively in a single centre, with a median age of 53 (range, 15-66) y. Diagnoses included 39 NHL (55%), 17 Hodgkin lymphomas (24%), 12 CLL (17%) and 3 myeloma (4%). PBSCs were used as stem cell source in 66 patients (93%), while 5 patients (7%) received classical bone marrow. A MRD was used in 43 cases (61%) and a MUD in 28 cases (39%). With a median follow-up of 43 (range 3.7-85) months after allo-SCT, all patients, but one (from the FTBI group) engrafted. In the FB1A group, the grade 3-4 aGVHD rate was 20.5%, the chronic GVHD rate was 32%, the relapse rate was 23% and the TRM rate was 25%.

In the FTBI group, the rate of grade 3-4 aGVHD rate was 44% (P=0.03 in comparison to the FB1A group), the chronic GVHD rate was 52% (P=0.09), the relapse rate was 15% (P=NS) and the TRM rate was 37% (P=NS). At 2 years, overall survival was 66% (95%CI, 51-78%) in the FB1A group versus 55% (95%CI, 36-73%) in the FTBI group (P=NS). Disease-free survival was also comparable between both groups (at 2 years, 59% in the FB1A group, vs 48% in the FTBI group, P=NS). In a Cox multivariate analysis for OS or DFS, the type of RIC regimen was not significantly associated with outcome.
These results suggest that in lymphoid malignancies, a RIC regimen including Fludarabine, ATG and low dose busulfan (4 mg/Kg total dose) is a valid alternative to the classical Fludarabine and low dose TBI-based RIC regimen with a favorable toxicity profile and efficient disease control.

P969

Phase II prospective multi-centre study of treosulfan-based conditioning in allogeneic HSCT for haematological malignancies from 10/10 HLA identical unrelated donor

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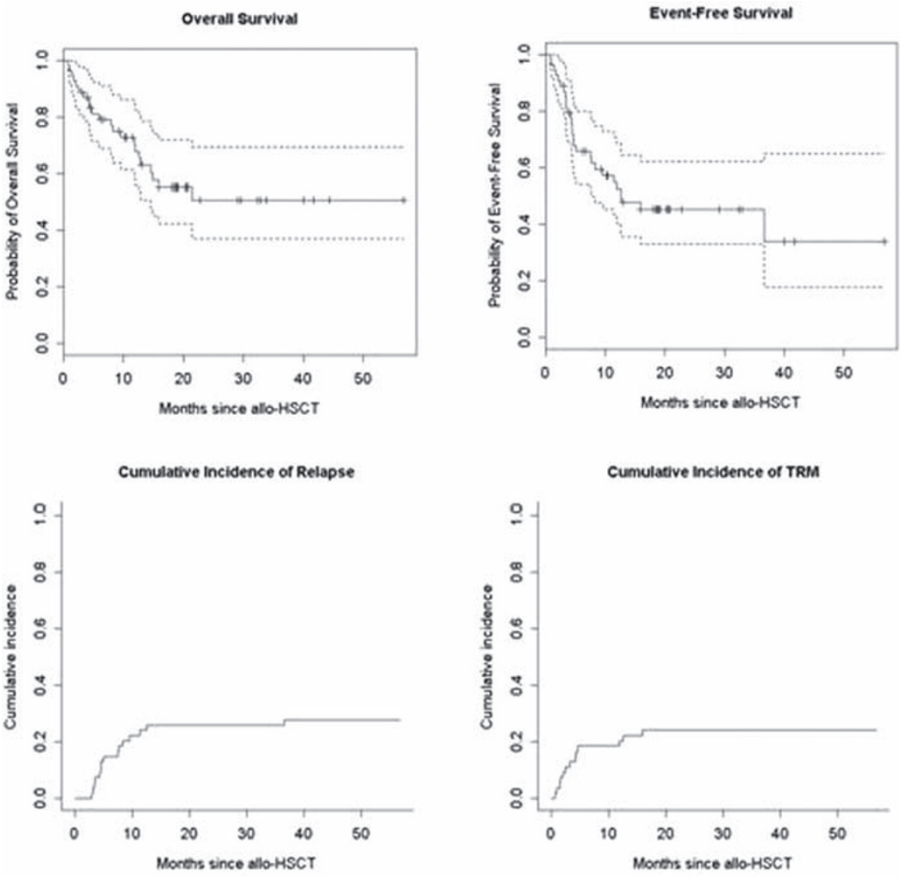
To evaluate the toxicity and efficacy of a RIC regimen including Treosulfan, followed by allo-HSCT from a 10/10 HLA identical unrelated donor, we conducted a prospective study in adult patients presenting a hematological malignancy in need of allo-HSCT. The conditioning included: Treosulfan 12 g/m²/day iv (day-6 to day-4), fludarabine 30mg/m²/day iv (day-6 to day -2) and ATG 2.5 mg/kg/day (day-2 to day-1). Peripheral stem cells were used as HSC source. We included 56 patients, 30 (54%) males and 26 females with a median age of 57 years (18-65.5). There were 29 AML (14 in CR1, 14 CR2 & 1<CR), 8 MDS (1CR1 and 7<CR) and 1 CML in CR1], 9 MM in PR, 6 CLL (2CR1 & 4PR) and 3 ALL (1CR1 & 2CR2)]. Among 45 explored for cytogenetics, 23 (51%) were normal and 22 with poor prognostic. Before transplantation, two patients did not receive any previous treatment, 21 received 1 line, 22 two lines and 11 > 2 lines, 49% of patients were sex-mismatched. For CMV, 43% were -/-, 25% +/+, 28% ± and 1% -/+. For ABO matching, 24% had major incomp. & 24% minor incopm. The median interval diagnosis-allograft was 15 months (4-168). Fifty-four (96%) patients engrafted with a median time to neutrophils and platelets recovery of 16 days (4-86), 11 (4-82) respectively, 17 patients developed aGVHD grade ≥ II with a cumulative incidence at 3 months of 31% (25-38). The cumulative incidence of cGVHD was, at 12 months: 32% (25-39) limited and 6% (2-10) extensive; at 18 months: 34% (27-47) limited and 8% (5-12) extensive. After a median follow-up of 13 months (1-57), the median OS was not reached with a 3 years probability of 52% (38-71). The median time of EFS was 15 months (8 - 57) with a 3-years probability of 47% (35-64). The cumulative incidence of relapse at 3 years was 25% (19-31) and the cumulative incidence of TRM at 12, 18 and 36 months was 20% (16-27), 23% (16-29) respectively. At the last follow-up, 22 patients died, 7 due to relapse and 15 due to TRM. Patients with active cGVHD seem to benefit for the GVL effect on OS(HR=0.2 (0.1-0.6) p=0.002). The multivariate analysis showed: a negative significant impact of both minor ABO incompatibility (p<0.001) and CMV±(p=0.01) on OS, a negative significant impact of patients <CR (p=0.03) on relapse. Treosulfan appears to be a good choice for conditioning with very promising results in terms of OS, relapse and TRM and can offer the desired low toxicity of RIC with full intensity antileukemic activity.



P970

Outpatient management of haematopoietic stem cell transplantation with reduced-intensity conditioning regimen: a single-centre retrospective study of 63 patients

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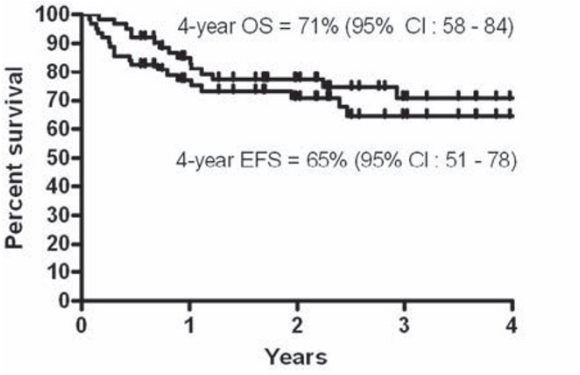
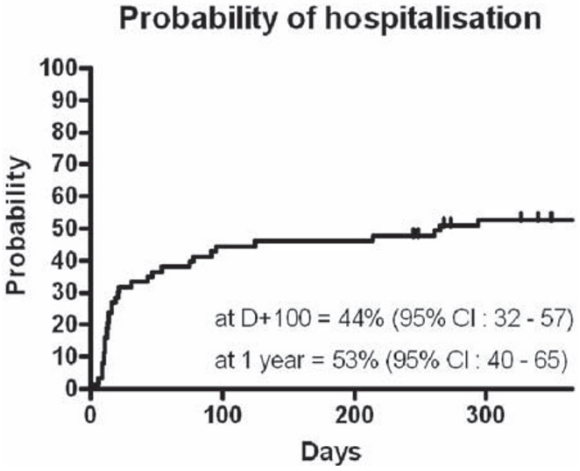
Increasing use for more than 10 years of RIC for allogeneic haematopoietic stem cell transplantation (SCT) has modified spectrum of indications and procedure. Decrease of short-term toxicity have led us to engage a program of outpatient management. We report a retrospective study of 63 consecutives patients (pts, median age = 52, 24-68) who underwent SCT between 2002 and 2009 after RIC (Fludarabine 30 mg/m^2 D-4 to D-1, Busulfan 4 mg/kg PO or 3.2 mg/kg IV D-4 and D-3, Thymoglobuline® 2.5 mg/kg D-4 and D-3) and intended to be managed as outpatients with planned visits in day hospital two to three times a week until D+28 (physical exam, blood monitoring, Xray and transfusion when appropriate). Stem source was PBSC in 60 cases (BM for 3 after refusal or failure of PBSC harvest). Donors were HLA-id = 30, MUD 10/10 = 26, 1 MM MUD 9/10 = 7. Diagnosis were: 54 AML, 5 multiple myeloma, 1 MDS/MPS, 1 DLBCL, 1 ALL, 1 CLL; 54 pts were standard risk (CR1 or 2) and 9 high risk (PR, refractory, untreated relapse). All pts except one engrafted: median duration of neutropenia < 0.5 giga/l was 6 days (0-18) and thrombopenia < 20 giga/l was 0 day (0-35). Patients received a median of 3 RBC units (0-25) and 0 platelet concentrate (0-24). With a median of follow up of 24.3 months, 4-year OS, RFS and EFS were respectively 71%, 66% and 65%. Probability of grade II-IV acute GvHD at Day+100 was 17%. The 2-year probability of moderate to severe chronic GvHD was 48%. No patient died because of outpatient management (such as septic complication during neutropenia or hemorrhage during thrombopenia) and the 4-year NRM was 10%. At Day+100 post-SCT, 27/63 (43%) had to be re-admitted as in-patients for a total number of 36 hospitalizations. The major cause of hospitalization was infection (21 cases) including 13 cases of febrile neutropenia; other cases included GvHD (7) relapse (4), EBV lymphoproliferative disease (1), others (3). The median time of first re-hospitalization was 17.5 day with a median duration of 8.5 days (3–28). Median hospitalization until D+100 was 9 days (6-42). Costs were evaluated for 24 patients from 2007: median was 28 k€ (16-78) to be compared to 123 k€ (33-177) for 8 patients managed as inpatients after myeloblative RIC (Busulfan same dose D-5 to D-3). These results support broader use of ambulatory SCT after RIC with good safety for patients, better use of inpatient facilities and likehood of decreased costs.





P971

Increased anti-thymocyte-gGlobulin dose in reduced-intensity conditioning did not increase the transplantation-related toxicity in patients with myeloid malignancies undergoing allogeneic stem cell transplantation from HLA-sibling donor

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Background: Reduced intensity conditioning regimen (RIC) with Fludarabine, oral Busulfan and 1 day of anti-thymocyte globulin (ATG) (FBA1) was previously reported to be effective and well tolerated. The aim of this study was to evaluate the outcomes of patients with myeloid malignancies undergoing to allogeneic transplantation (ALLO-SCT) with FBA1 or two days of ATG (FBA2).

Methods: We analyzed 75 patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) undergoing Allo-SCT from a HLA-identical sibling donor from 2000 to 2009. RIC consisted of fludarabine (30 mg/m² over 5 days), oral or IV busulfan (8 mg/kg total dose) and rabbit ATG 2.5 mg/kg 1 day (FBA1; n=53) or 5 mg/kg (FBA2; 2.5 mg/kg/day over 2 days) (n=22). Secondary AML/MDS, adverse cytogenetic and/or transplantation not in complete remission were criteria to define high risk disease (HR).

Results: Median age was 50 years [18-70]. Patients in FBA2 were older (median age of 60 years vs 49 years, (p=0.032) and treated more recently. No difference in terms of diagnosis and status before ALLO-SCT were observed.

Acute GVHD (grade 2-4) incidence was not statistically different between the two groups (30% and 14% in FBA1 and FBA2, respectively, p=0.12). Extensive chronic GVHD was significantly reduced in FBS2 (59% vs 20%, p=0.002).

With a median follow up of 49 months [4-110], for all patients, the 1- and 5-year OS were respectively 74% and 59%. 1- and 5-year non relapse mortality (NRM) was 18 % and 22%, respectively. NRM was adversely associated with grade 2-4 acute GVHD (53% versus 24%, p<0.01). Relapse occurred in 18 patients (29%), at a median time of 4.6 months [1-39]. The 1- and 5-year OS were 74% and 59% versus 70% and 62%, the 1- and 5-year NRM were 17% and 19% versus 15% and 22%, in FBA1 and FBA2, respectively (p=NS).

In univariate analysis, only HR disease was associated significantly to a higher relapse risk compared to standard risk disease (41% vs 16%, p=0.043). ATG dose did not influence OS, relapse incidence, and NRM.

Conclusions: In this retrospective study, ATG 5 mg in RIC regimen reduced significantly the incidence of chronic GVHD compared to lower dose, after ALLO-SCT from HLAid sibling donor. The risk of relapse was not enhanced and the survival was not statistically different. Only the risk of disease influenced the relapse incidence. These encouraging results deserve a prospective randomized evaluation.

P972

Treosulfan-based conditioning regimen allows an early full donor chimerism after related and unrelated transplant with low toxicity. Results from a prospective single-centre study

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Allogeneic Stem Cell Transplantation (ASCT) is the only curative treatment for many haematological disease. Toxicity of normal conditioning regimens could rule out patients (pts) from the chance of having access to the cure. We investigate the efficacy of a Treosulfan-based conditioning regimen for ASCT. Treosulfan is a bifunctional alkylating agent with a reduced extra-haematologic toxicity profile but with a strong cytotoxic action. We transplanted in our centre, since 2004, 117 pts with a median age of 49 years (21-69), 26% of them were older than 60. Diagnosis were: AML=40, ALL=14, MDS=16, CML=5, MPD=6, Lymphoma=30, MM=5 and BM failure=1; 58 pts were transplanted in remission, 59 with active disease. Median follow up was 36 months (2-72). 68 pts were transplanted from an unrelated donor (UD), 49 with matched sibling donor. Conditioning was based on Treosulfan (14 g/mq for 3 days) and Fludarabine (30 mg/mq for 5 days). In vivo T and B-cell depletion was performed respectively by ATG-Fresenius (10 mg/kg for 3 days) and Rituximab (single 500mg dose) only in pts receiving an UD. GvHD profilaxys consisted of Cyclosporine A and, excepting cord blood (CB), a short course of Methotrexate. 100 pts were transplanted with peripheral blood stem cell, 5 with bone-marrow and 12 patients with CB. Median Sorror score was 2 (0-7), 39 pts had a score of 0. At day 60 neutrophil recovery was obtained in 94% of pts in a median time of 17 days (9-36). Platelets recovery was obtained in 88% of pts, median time was 15 days (8-77). Chimerism at day 30, in VNTR analysis on bone marrow, was full-donor in 89%, mixed in 10% of pts and only 1 pt had a full-host chimerism. In sorted CD3+ and CD13+ cells chimerism was: 13% mixed and 87% full donor, 2% mixed and 98% full donor respectively. Only 12% of pts experienced a >2 regimen-related toxicity, according to CTC score, and the most frequent toxicity was a transient rise of bilirubin (82% of cases). Transplant related mortality (TRM) at 3 years was 21±4%. In multivariate analysis factors that increase TRM were: UD (p=0.016 HR=6.053), active disease at transplant (p=0.017 HR=3.404) and age >60 years (p=0.041 HR=2.464). 3 years Overall Survival (OS) was 62±5%. Forty-five pts died after transplant, 47% for relapse and 53% for transplant related causes (46% infections, 21% GvHD). Our results demonstrate that Treosulfan is a very effective drug, that allows an early full-donor engraftment with low extra-haematological toxicities.

P973

Outcome of reduced-intensity allogeneic haematopoietic stem cell transplantation in acute lymphoblastic leukaemia: a British Society for Blood and Marrow Transplantation study

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Allogeneic haematopoietic stem cell transplant (allo-SCT) is effective in adult acute lymphoblastic leukaemia (ALL) but high risk patients experience excessive transplant related mortality with myeloablative conditioning. We therefore investigated the outcomes of reduced intensity conditioned (RIC) allo-SCT for ALL reported to the British Society for Blood & Marrow Transplantation. 76 patients (40 female) underwent allo-SCT from matched related (n=34) or unrelated (n=42) donors between 1993 & 2009. Median age was 41y (range 16-58). Philadelphia chromosome (Ph) was present in 26 of 59 patients (unknown = 17). 67 patients were in complete remission (CR1=36, CR2 or subsequent CR=26, CR (unspecified)=5) and 8 patients had active disease (1 unknown). This was the first allo-SCT in 50 patients (66%), second in 25 patients (33%) and third in 1 patient (1%). Indications for RIC were age (n=20), co-morbidity (n=11), required by local protocol (n=17), prior MAC HSCT (n=14) and other/unknown (n=14). In 36 patients with available data 11 (31%) had required ≥2 lines of chemotherapy prior to achieving first CR. Fludarabine with melphalan (n=46) or with cyclophosphamide (n=9) were the commonest conditioning regimens. Alemtuzumab was used in 46 (61%) transplants. Myeloid engraftment occurred in 70 patients (92%). 17 patients (22%) developed grade II-IV acute graft-versus-host disease (GVHD). In 61 patients surviving beyond 100d chronic GVHD occurred in 20 (36%, data unavailable for 6). With median follow-up of 23 months 2y overall/leukaemia-free survival (OS/LFS) was 35% (95%CI = 24-47%) and 30% (19-42%) respectively. Non-relapse mortality (NRM) and relapse incidence (RI) were 28% (17-39%) and 42% (30-54%) respectively. Relapse occurred in 29 patients, relapse sites were: medullary (n=18), extramedullary (n=4) or both (n=2), data unavailable (n=5). In univariate analysis neither Ph+ disease nor alemtuzumab were associated with impaired outcomes. Recipient male sex, the presence of cGVHD and fewer lines of treatment to CR1 were associated with improved OS and LFS. cGVHD was associated with reduced RI (HR 0.4, p=0.002) suggesting the importance of graft-versus-leukaemia effect. In multivariate analysis of patients with available data (n=36) only fewer treatment lines associated with improved OS & LFS. RIC allo-HSCT enables medium-term disease control in chemosensitive adult ALL with equivalent outcomes in Ph positive and negative disease.

P974

Feasibility of allogeneic stem cell transplantation conditioned with thymoglobulin and reduced-intensity total body irradiation

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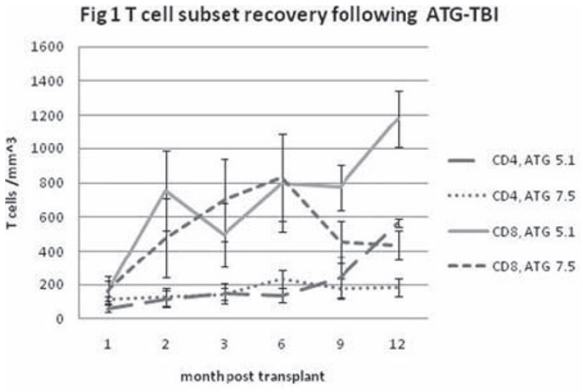
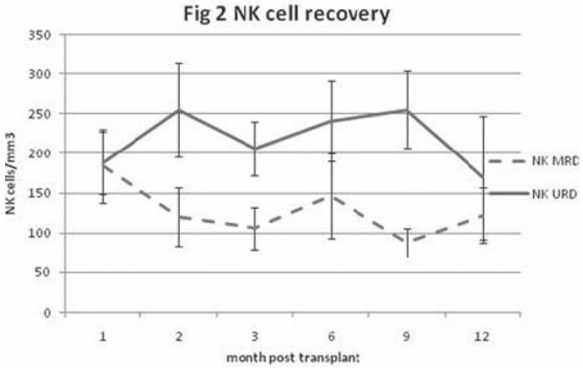
In vivo T cell depletion with rabbit anti-thymocyte globulin (r-ATG, Thymoglobulin, Genzyme Inc.) reduces the risk of graft vs host disease (GVHD), however poor T cell reconstitution seen with current schedules results in a high incidence of opportunistic infections and relapse following stem cell transplantation (SCT). We report data on engraftment and immune reconstitution in patients randomized to receive conditioning with either 5.1 (n=9) or 7.5 (n=10) mg/kg of r-ATG in divided doses between days -9 and -7, followed by 450 cGy total body irradiation (TBI). GVHD prophylaxis is with tacrolimus and mycophenolate mofetil . These patients are heavily pre-treated; 12 received SCT from unrelated donors (URD), 7 from matched related donors (MRD). Diagnoses include MM (6), NHL (6), (2), CLL/PLL (3/2), AML & MDS (1 each). Median patient age is 58 years. All patients have demonstrated sustained complete myeloid donor chimerism at 1-12 months post transplant. T cell chimerism is predominantly donor derived (≤10% recipient DNA in CD3+ cells: at day 90 (OR: 1.25, 95% CI: 0.15, 10.70) and at day 180 (OR: 0.33, 95% CI: 0.03, 4.19). T cell reconstitution is comparable in both arms (Fig 1); there is a trend towards more robust NK cell recovery in URD recipients (Fig 2). With a median follow up of 325 and 502 days in the ATG 5.1 and 7.5 arms, 78% and 80% of the patients are alive respectively. Three patients in the ATG 5.1 and one in the ATG 7.5 arm developed ≥ grade II acute GVHD (OR: 4.80, 95% CI: 0.38, 59.89) and chronic GVHD was seen in 0/8 vs 6/9 evaluable patients (OR: 0.03, 95% CI: 0.00, 0.73). Three patients have relapsed (1/8 vs 2/9; OR: 0.50, 95% CI: 0.04, 6.86); this represents a marked improvement in outcomes compared with a historical cohort of

ATG 10 +450 cGy TBI given on a similar schedule (8/14 relapse; Toor et al, 2008). CMV reactivation developed in 7/11 seropositive pairs. In conclusion conditioning with two different doses of Thymoglobulin and reduced intensity TBI results in equivalent lympho-hematopoietic engraftment in older patients receiving MRD and URD transplants, with an acceptable risk of acute and chronic GVHD. ATG used in the conditioning promotes durable NK cell recovery in URD recipients with possible implications for KIR mediated anti tumor activity. ATG 7.5 patients receiving a URD blood stem cell graft appear to have the best outcomes in terms of progression free survival amongst the subgroups.





P975

Impact of high-resolution HLA typing HLA- A, -B, -DRB1 in adult patients with reduced-intensity cord blood transplantation

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To determine the impact of high-resolution (HR) HLA typing with outcomes after UCBT DNAs of 172 pairs were analysed for HLA class I and class II mismatches (MM) based on HR typing. This study included adult patients with hematologic malignancies who underwent RI-CBT as their first allogeneic SCT at Toranomon Hospital between January 2005 and December 2008 consecutively. Patients who had active serious infection or showed an Eastern Cooperative Oncology Group performance status of 3 or 4 before transplantation were not eligible for this study. For A, B, and DRB1 based on HR typing the following MM occurred: no MM 3(2%), one MM 12(7%), two MM 67(39%), three MM 58(34%), four MM 23(13%), five MM 9(5%) in GvH direction. And in HvG direction , no MM 4(2%), one MM 13(8%), two MM 62(36%), three MM 56(32%), four MM 28(16%), five MM 9(5%). The degree of HLA MM in the GvH direction was associated with engraftment kinetics, whereas no statistically significant association was observed with the degree of HLA MM in the HvG direction; There was a trend that MM in class I HR were associated with neutrophil recovery. There was no significant association between number of MM (HR) for both HLA-A, -B and -DRB1 aGvHD grade II–IV. No significant correlation was found between numbers of HLA-MM on the HR level with 2-year survival.
High-resolution HLA mismatching did not affect OS or DFS in adult patients with reduced-intensity cord blood transplantation.

P976

Myeloablative conditioning with intravenous busulphan in a single daily dose and fludarabine for HLA-identical sibling allogeneic HSCT in myeloid malignancies

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Background: There is a need to improve the conditioning regimens for allogeneic HSCT, reducing the regimen related toxicity while maintaining the anti-leukemic effect. The combination of myeloablative doses of intravenous busulphan (BU) with fludarabine (F) may contribute to achieve these goals, since they have been reported to have an improved safety profile over BUCY.
Objective: We aimed to test the efficacy and safety of this combination (BUF), with a single daily dose of BU, since prior pharmacologic and clinical studies support its safety compared the with standard 4-daily doses.
Patients: Eighty seven consecutive adult patients undergoing HLA identical sibling allogeneic HSCT for myeloid malignancies were included in this report. Their main clinical characteristics are shown in Table 1. Conditioning regimen consisted in BU, one daily IV infusion (3.2 mg/kg/d) for 4 days (total dose 12.8 mg/kg), combined with F, 40 mg/m2 daily (total dose 160 mg/m2). GVHD prophylaxis consisted in cyclosporine and methotrexate. Antimicrobial and other supportive measures were followed at each institution policies. Donor graft source was peripheral blood (PB) in 57% and bone marrow (BM) in 43% of cases. Median CD34+ cells infused were 4.7 millions/kg (range 0.6-17.8).
Results: All but one patient engrafted, with a median of 15 days (range 8-34) to >0.5 x10E9 PMN/L and 12 days (range 7-46) to >20 x10E9 platelets/L. Most of the observed toxicities (Bearman scale) were grade 1 (Table 2). Major toxicity was mucositis. There were 3 grade-2 VOD cases (3.8%), all of which resolved. Acute GVHD grade 2-4 incidence was 24.4% with a

Patients	**87**
Age: median (range) years	46 (19-74)
Patients aged >55 years	23 (27%)
Male gender	44 (58%)
Disease	
AML	50 (59%)
MDS Intermediate/High risk	15 (18%)
Secondary AML	14 (18%)
Myeloproliferative disorder	6 (7%)

Reg related toxicities	Cases	Grade 1	Grade 2	Grade 3
Mucositis	61	19	32	10
GI tract	17	13	4	
Liver	19	16	2	1
Hepatic SOS (VOD)	*4*	*1*	*3*	
CNS	6	2	4	
Cardiac/Pulmonary	2	2		
Acute GVHD	34	15	19 Grades 2-4	

Data collected from 78/87 patients

median of 33 days (range 15-87) to GVHD onset. The day-100 mortality was 5.8% (5 cases). There were 2 cases of secondary graft failure which preceded leukemia relapse. At the time of this interim analysis, the median follow-up is 16 months (range 3-51). Crude survival data showed 69 (79.3%) patients remaining alive and 69 (79.3%) relapse free. Overall, 17 patients have died, 12 relapse-related (13.8%), 4 due to GVHD or infection (4.6%) and 1 (1.1%) due to other illness.
In conclusion, BUF regimen in the HLA identical allogeneic HSCT setting provides an excellent tumor control and a reduced regimen related toxicity, day-100 mortality and non-relapse related mortality.

P977

Busulfan-based reduced-intensity regimen before allogeneic stem cell transplantation: tolerance of three days intravenous and fractionated busulfan

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Reduced-intensity regimen (RIC) using Bu has proven its efficacy and its good safety (Blaise et al1). Bu usual dosage was 3,2 mg/kg/day during two days.Higher dose of Bu may be useful to improve disease control in some patients in partial response or stable disease with high minimal residual disease. We report our results in 19 patients who received this conditionning with increased dose of Bu.
Since 2008, 19 patients presenting acute myeloid leukaemia (6), non Hodgkin's lymphoma (4), chronic myeloid leukaemia, chronic lymphocytic leukemia (4) and idiopathic myelofibrosis (2) received RIC and allogeneic (RIC-Allo) transplantation. The median age was 58 years (49-65). Disease status at RIC-allo was first complete remission in 8 cases, second complete remission in 2 cases, partial response in 5 cases and stable disease in 3 caes.
RIC consisted of Fludarabine 40 mg/m²/day for 5 days, Bu intravenously fractionated in four doses 3.2 mg/kg/day for 3 days (4 days for one patient) and Thymoglobulin 2.5 mg/kg/day for 2 days (1 dose for one patient, 3 doses for one patient).
The majority of donors was unrelated match (10/10 for 11 pts, 9/10 for 3 pts). Immunosuppression was based on ciclosporine in all patients and mycophenolate mofetyl was added in case of unrelated donor. All 19 patients received peripheral stem cell. The median number of infused CD34 cells was 6.10^6 CD34/mm3 (4.1-12.2);
The median follow-up was 17 months (6-32). All pts but one engrafted. None sinusoidal obstructive syndrome was noted. Two patients died from acute GVHD, one patient from progressive disease, one patient from late pulmonary infection with pneumocystis carinii. All but one were in complete response at day 100.
Acute GVHD (gradeII-IV) occurred in six patients and all but two resolved with corticosteroids. Chronic GVHD was noted in 10 pts (limited in7 pts, obliterans bronchiolitis in one case, extensive cGVHD in three cases).
Liver toxicity WHO grade III- IV was seen in 7 pts but was reversible. Mucositis WHO grade III was noted in 3 pts.
We showed as Richardson et al2 that additional day of Busulfan in RIC-Allo is well tolerated and permits more cytotoxic weight in high-risk of relapse patients. Prospective comparative studies are need to show the real antitumoral effect of adding dose of Busulfan.
1-Blaise D et al. Exp Hematol 2010 38(12) ;1241-50.
2-Richardson DS et al. Bone Marrow Transplant 2009.

P978

Outcome of patients aged ≥60 after allogeneic haematopoietic cell transplantation: age has no impact on survival

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The introduction of reduced intensity conditioning (RIC) regimens enabled successful hematopoietic cell transplantation (HCT) in elderly patients but the impact of age on outcome has not been evaluated extensively.
We therefore retrospectively analyzed 119 consecutive patients (f=48, m=71) aged ≥60 who received allogeneic HCT 2000-2010 at our institution. Median age of the patients was 65 years (range, 60-76). Patients were grouped in two cohorts: group 1 aged 60-65 years (n=60, median age=63) and group 2 aged 66-76 years (n=49, median age=68). Diagnoses were acute leukemia (AML=70, ALL=1), myelodysplastic syndrome (n=17), osteomyelofibrosis (n=7), Non-Hodgkin lymphoma (n=9), multiple myeloma (n=9), aplastic anemia (n=1), chronic myeloid (n=3) and chronic lymphatic leukemia leukemia (n=2). At time of HCT 40 of the patients were in complete (CR), 79 in partial remission (PR) (group 1: CR=21, PR=45, group 2: CR=19, PR=34). Conditioning regimens were grouped in high (TBI/Bu+Cy, n=5), intermediate (FLAMSA, Flu/Mel/BCNU, n=33), low (FLU+alkylans, n=53 and minimal (2GyTBI/Flu, n=28) intensity. Intermediate and low intensity conditioning was particularly used for high risk patients in PR (65/79, group 1=38, group 2=28 while minimal intensity conditioning was mostly used for patients in CR (18/28, group 1=8, group2=10). 21 of the patients had a preceding HCT, 15 in group 1. 24 patients were transplanted from matched related (MRD), 49 from matched unrelated (MUD) and 46 from mismatched unrelated donors (MMUD). Kaplan-Meier-estimated 3-year overall survival (OS) 32% in group 1 and 65% in group 2 (p=0.04). Non-relapse-mortality was 38% in group 1 and 19% in group 2 (p=0.009). Incidence of relapse related death was 23% in group 1 and 17 % in group 2.
Table 1 describes Kaplan-Meier estimated 3-year-OS and statistical univariate analysis by log-rank test in the different subgroups.
Older age alone had no negative impact on the outcome of allogeneic HCT. Chronic GVHD and the use of MUD had a positive influence on survival. Our data indicate that the regimen used should be tailored to disease risk and patient performance status rather than age.

Table 1.

3-year OS (in%)		All		Group 1 Age 60-65		Group 2 Age 66-76	
Remission	CR	57	p=0.25	38	p=0.45	83	p=0.11
	PR	38		28		62	
Conditioning	high	0	p=0.03	0	p=0.02	-	p=0.45
	intermediate	40		19		57	
	low	53		48		65	
	minimal	46		17		69	
Donor	MRD	0	p=0.001	0	p=0.03	63	p=0.76
	MUD	58		43		74	
	MMUD	48		42		56	
GVHD acute	no	46	p=0.003	35	p=0.005	61	p=0.23
	≥II	18		13		33	
chronic	no	37	p=0.10	31	p=0.90	52	p=0.10
	limited	60		19		72	
	extensive	55		44		100	

P980

Similar outcome after related or unrelated allogeneic stem cell transplantation following reduced conditioning with fludarabine, busulfan, and antithymocyte globulin

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The comparison of outcome of in vivo T cell depleted reduced-intensity allogeneic stem cell transplantation (allo-SCT) from related or unrelated donor remains a matter of debate. To investigate the effects of donor type in this setting, we conducted a retrospective analysis in our centre.

Seventy-eight patients were selected based on a reduced conditioning with fludarabine, busulfan, and rabbit antithymocyte globulin (5mg/kg). Allo-SCT were performed between january 2003 and july 2010. Median age was 56 years (range, 16 to 67). Diseases were distributed as follow: acute myeloid leukemia (n=32), acute lymphoid leukemia (n=4), lymphoma (n=20), myelodysplastic syndrome (n=8), myeloma (n=9), chronic lymphocytic leukemia (n=3), myeloproliferative syndrome (n=2). Donors were matched related (n=35), matched unrelated at the allele level (n=26), or mismatched unrelated (n=17, 11 single antigen mismatches and 6 single allele mismatches). Status of disease at transplant were distributed as follow: CR1 (n=31), ≥ CR2 (n=19), PR1 (n=2), ≥ PR2 (n=9), refractory disease (n=12), and untreated (n=5). Peripheral blood was the main source of stem cells (n=73) and the median number of CD34+ cells infused was 6.3 x 10^6 (range, 1.9 to 18.6).

With a median follow-up of 22 months (range, 3 to 84) the 2-year overall survival (OS), disease-free survival (DFS), non-relapse mortality (NRM) and incidence of relapse were 70%, 61%, 18% and 25%, respectively. Twenty-five patients have died from the following causes: disease (n=13), acute GvHD (n=1), multi-organ failure (n=1), acute respiratory distress syndrome (n=2), septic shock (n=6), bronchiolitis obliterans (n=1), and encephalitis (n=1). Multivariate analysis indicated that refractory disease adversely affected OS, DFS, and incidence of relapse. Multivariate analysis was unable to identify a predictor of increased risk of NRM. The cumulative incidences for grade II-IV aGvHD, grade III-IV aGvHD and extensive chronic GvHD were 30%, 12%, and 29%, respectively. Multivariate analysis indicated that grade II-IV aGVHD and extensive chronic GvHD were both favoured by age ≥ 60 years and that grade III-IV aGVHD was favoured by refractory disease. The effect of donor type was non significant for all criteria.

These data suggest that allo-SCT from related or unrelated donor following a reduced conditioning with fludarabine, busulfan, and rabbit antithymocyte globulin result in similar outcome regarding OS, DFS, NRM, relapse and GvHD.

P981

Fludarabine with high-dose cytarabine and sequential reduced-intensity conditioning with allogeneic stem cell transplantation in 24 patients with advanced lymphoid malignancies: efficacy and outcomes

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Background: Sequential use of chemotherapy and reduced-intensity conditioning (RIC) for allogeneic stem cell transplantation (SCT) in high-risk leukemia patients (pts) represents a promising approach (Schmid et al., JCO 23, 2005: 5675-87). We examined the toxicity and efficacy of this therapy at cohort of 24 pts with high-risk lymphoid malignancies, we used the analogical protocol without administration of amsacrine.

Methods: High-risk was defined by progressive or refractory disease (n = 7), disease on the second or third remission (n = 16), or ALL with bcr/abl positivity (n = 1). Fludarabine (30 mg/m²) and cytarabine (2 g/m²) for 4 days (FC) were used for cytoreduction. After 3 days of rest, RIC consisting of 4 Gy TBI, anti-thymocyte globulin (ATG-Fresenius) 10-20 mg/kg/day for 3 days, and cyclophosphamide 40-60 mg/kg/day for 2 days followed.

We analyzed 24 pts (ALL, n=5; CLL, n=8; NHL, n=11) undergoing chemotherapy and RIC SCT in our centre from August 2006 to May 2010. Disease status before SCT was: CR1, n=1; CR2, n=7; CR3, n=1; PR2, n=7; PR3, n=1; refractory/progressive disease, n=7. Types of donors and used grafts were as follows: HLA identical sibling, n=9; unrelated donor, n=15, PBSCs, n=22, BM, n=2. Median age of pts was 49 years (range 25-62).

Results: The median time of neutrophil engraftment (above 0.5x10E9/L) was 17 days, all pts engrafted. The most frequent toxicities were grade III/IV infections according to common toxicity criteria in 16 of 24 pts and gastrointestinal toxicities (grade III in 7 of 24 pts).

Incidence of acute GVHD was evaluated in 23 pts: 61% (14/23) of pts had GVHD (grade I+II in 11 pts, grade III in 3 pts). Incidence of chronic GVHD was evaluated in 20 pts, 45% (9/20) of pts had GVHD (limited in 5 pts, extensive in 4 pts).

Nonrelapse mortality (NRM) after 100 days and 1 year was 8% and 21%. Causes of death were refractory GVHD (n=2) and septic shock with multiorgan failure (n=3).

Treatment response was evaluated in 22 pts: remission was achieved in 21 pts (95%), only 1 pt had progression.

With median follow-up from SCT 14 months (range 1-49), 63% of all pts (15/24) were alive (14 pts in remission, 1 pt with relapse), 9 pts died (5 deaths from NRM, 4 deaths from relapse or progression), 5 relapses (24%; 5/21) occurred. Median follow-up for 15 survivors was 20 months (range 4-49).

Conclusion: FC-RIC protocol seems to be feasible and effective alternative for pts with advanced lymphoid malignancies with acceptable toxicity.

P982

Long-term survival of 2Gy TBI and fludarabine as the RIC conditioning in the treatment of repeated relapsed lymphoma

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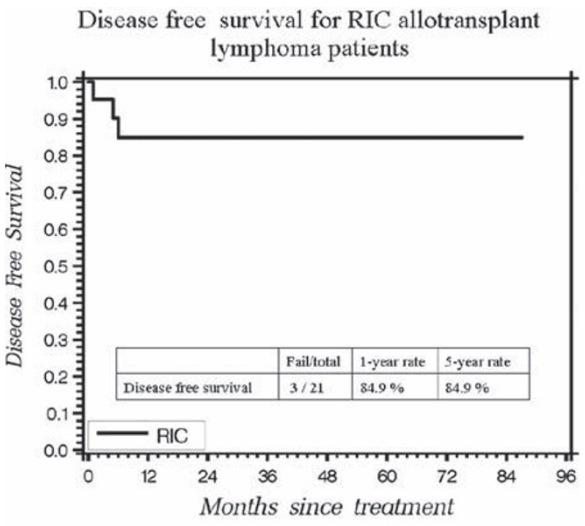
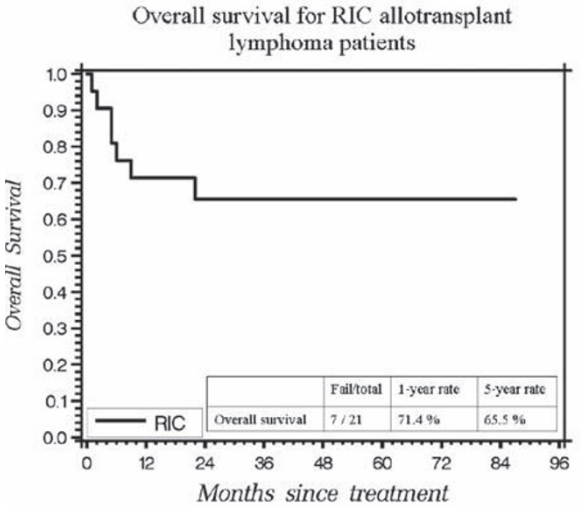
Background: For all chemosensitive, indolent lymphoma got remittent and repeated relapsed. Conventional chemoimmunotherapy or even high dose chemotherapy plus autologous haematopoietic stem cell transplantation could not cure this kind of disease. Myeloablative allotransplant offers the promising cure but had greater transplant related morbidity and mortality. Therefore, reduced-intensity allotransplant is the curative treatment of choice with acceptable risk.

Method: We analyzed the long-term outcome and cause of treatment failure of our repeated relapsed lymphoma patients underwent RIC allogeneic haematopoietic stem cell transplantation. All the patients have had undergone at least 3 lines of previous treatment when complete remission achieved and at least 2 lines of treatment when just partial remission or refractory disease before allotransplant performed. Conditioning regimen is 2Gy TBI plus fludarabine 90mg/m² and immunosuppressants includes cyclosporin plus MMF. Stem cell source could be from related or unrelated donors.

Results: Between 2003 and 2010, 21 repeated relapsed lymphoma patients underwent RIC allotransplant at our institute with median age 43.4 years (range 24~60) and M/F 14/7. The subtypes of lymphoma included follicular lymphoma 7, mantle cell lymphoma 5, diffuse large B cell lymphoma 2, Hodgkin lymphoma 3, CLL/SLL 1, and T-cell lymphoma 3. Nine patients (42.9%) were in CR2 or CR3 and the other 12 patients (57.1%) were in beyond or relapsed/refractory status of disease before allotransplant. Seven patients received stem cell from matched unrelated and the other 14 from matched related donors. The 7-year disease free survival was 84.9% and overall survival

was 65.5%. Seven patients died and 2 of them died of lymphoma relapse and 5 of them were attributed to non-relapse mortality with 2 patients died of infection and 3 died of GVHD. For 19 patients evaluable for GVHD, there were 8 grade II~IV (42.1%) with 4 grade III~IV (21.0%) acute GVHD and 3 had limited chronic GVHD (15.8%) and 9 extensive chronic GVHD (47.3%).
Conclusion: RIC allogeneic haematopoietic stem cell transplantation is the curative choice of treatment for repeated relapsed lymphoma patients but the extensive chronic GVHD is still a roadblock to be overcome.





P983
RIC-AlloSCTis associated with low NRM without increasing relapse risk in young, favourable cytogenetics, good risk patients affected by leukaemia and MDS
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Reduced-intensity conditionings (RIC) for allogeneic hematopoietic stem cell transplantation (AlloSCT) are usually used in non-fit or older fit patients (pts) as alternative to myeloablative conditionings (MAC). As a consequence, most of these pts are > 45 years old, since pts < 45 years old are likely to receive a MAC. Here we analyze a cohort of young pts (i.e. < 45 years old) who received a RIC for a hematological malignancy, in order to compare RIC-AlloSCT outcomes with those reported with MAC for similar populations.
Data on pts < 45 years old who received AlloSCT after a RIC conditioning at our Institution were collected. Diagnoses of acute leukemia (both AML and ALL), MDS, CML or other myeloproliferative syndromes were retained. Patients were classified according to the reason of RIC. Disease status at AlloSCT, cytogenetics at diagnosis and use of busulphan during conditioning regimen were also evaluated for final outcome.
A total of 45 transplants performed on 43 pts were identified, transplanted between June 2000 and August 2009; median age at transplant was 40 (18-45), median follow-up was 757 days (449-3236). Main characteristics are shown in table 1. OS, PFS, NRM and relapse incidence were 56%, 48%, 10% and 43% respectively. Grade 2-4 aGvHD occurred in 10 patients (25%) while severe, grade 3-4 aGvHD occurred in only two pts (5%). We observed a strong impact of cytogenetics on OS among pts with early disease (n=23, AL in CR1 or CR2), with survival rates 71% vs 17% and relapse 29% vs 67% for favourable and unfavourable group pts respectively (p=0.06 and p=0.12). Interestingly, no toxic deaths were observed among the 17 early disease and favorable cytogenetic pts. Use of busulphan during conditioning appeared to confer superior disease control (27% vs 74% and 35% vs 77% relapse rate in early and advanced disease group respectively) without affecting NRM that was similar in both group.
Present results confirm a low incidence of aGvHD and NRM among pts undergoing RIC-AlloSCT, despite a high relapse risk for AL with unfavourable cytogenetics. No impact of therapy/infectious complications before AlloSCT was observed. RIC could represent a valid alternative to MAC for those pts with early disease status at AlloSCT and favourable cytogenetics at diagnosis, with 71% OS, 29% relapse rate and 0% NRM in our series. Further approaches, investigating the role of increased busulphan doses on disease control are warranted and ongoing.

Variable		*N*	*%*
Conditioning	FludaTBI-based	10	22
	FludaBuATG-based	32	71
	Other	3	7
Donor	Sibling	40	89
	MUD 10/10	4	9
	MUD 9/10 (Mm DQ)	1	2
Cause of RIC	second HSCT	10	22
	Severe infection	7	16
	prior ASCT (BEAM)	13	29
	age >= 40	7	16
	other	8	18
Diagnosis	AML	28	62
	ALL	4	9
	CML	6	13
	MDS	6	13
	Myelofibrosis	1	2
Disease status at HSCT	AL CR1	17	38
	AL CR2	6	13
	Others	22	49
Cytogenetics			
Favourable*	CBF, t(15;17)	8	18
	normal caryotype	17	38
Unfavourable**	t(9;22), 5q-, mon7, complex	16	36
	other abnormalities	4	9

*patients with CBF and t(15;17) were in ≥ CR2 at transplantation
** t(9;22) was considered as unfavourable only in patients

P984

Treosulfan/fludarabine-based conditioning for allogeneic stem cell transplant in high-risk haematologic malignancies. A 5-years single-centre experience

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Allogeneic stem cell transplant (allo-HSCT) is a curative approach in hematologic malignancies. Reduced toxicity conditioning are explored to allow HSCT to a wider patient population. This study evaluated efficacy and toxicity of Treosulfan-Fludarabine in patients receiving match sibling or MUD transplant for advanced hematologic malignancies and in older patients not fitting for myeloablative treatment.
Since July 2005 to September 2010 fifty-five (55) consecutive patients (20 females, 35.males) entered this study. Mean age was 48 years (range,17-66). Underlying diseases were 28 acute leukemias (18 myeloid, 10 lymphoid), 10 MDS , 7 NHL, 4 HD, 2 IMF, 1 BC-CML, 1 MM, 1 CLL, 1 hystiocitic sarcoma). Nineteen (19) patients received previous autologous transplant, 2 previous allogeneic transplant; 5 patients suffered from a previous solid tumor. Mean HSCT-CI was 1 (range 0-7).
Twenty-eight (28) patients were in CR at the moment of the transplant; 8 were in 1st CR.
Conditioning consisted of Treosulfan 12-14 gr/mq for 3 days, Fludarabine 30 mg/mq for 5 days and Thiotepa 10mg/kg single day (in 13 patients). Cyclosporine-short MTX were used as GVHD prophylaxis and anti-Thymocyte globulin (Thymoglobulin or ATG-Fresenius) was used in patients receiving MUD transplants or PBSCs from an HLA identical siblings. Twenty-seven (27) patients received HSCs from HLA identical siblings and 28 from MUDs. Source of stem cells was bone marrow in 11 patients and peripheral blood stem cells in 44 patients.
Fifty-two (52) patients regularly engrafted; three high risk patients died before engraftment.
No conditioning related death was observed. Toxicity was mild–moderate in 12 patients, severe in 3. Acute GVHD was mild in 18 patients, moderate in 1, severe in 5 patients. Chronic GVHD developed in 19 patients (19/45); in 5 it was extensive (11%).
Thirty-one(31) patients are alive with a follow-up ranging from 2 to 61 months. Eighteen (18) patients relapsed and 12 died (21.8%) for progression of the disease. Twelve (12) patients died for TRM (21.8%).
Kaplan-Meier OS and DFS curves at 5 years reach 50% and 35%. The difference between OS and DFS survival is due to late relapses in 3 patients, now in remission after salvage therapy. They were 2 HR AML and 1 Ph positive ALL.
This experience underlines feasibility of Treosulfan based conditioning in a cohort of high risk patients. The relapse rate after transplant remains a crucial point probably requiring new strategy.

P985

Graft from HLA-identical sibling versus HLA-allelic-matched, unrelated donor (10/10) in patients undergoing allogeneic stem cell transplantation following reduced-intensity conditioning regimen

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Reduced-intensity conditioning (RIC) regimens before allo-SCT have emerged as an attractive modality to decrease transplant-related toxicity while preserving the graft-versus-tumor effect. One third of patients have an HLA-identical sibling donor. Stem cell grafts from unrelated donors are being increasingly used. We have reported, that in patients with standard-risk malignancy undergoing allo-SCT with CPM and TBI as conditioning treatment, marrow as a source of graft, and Cs-A plus short-course MTX as GVHD prophylaxis, there were no significant differences between the outcomes of patients receiving graft from siblings and those from unrelated fully HLA-matched donors (Yakoub-Agha et al, JCO 2006).
Here we report a retrospective study of 58 consecutive patients who received RIC allo-CST. Donors were HLA-identical sibling (n=35) and unrelated molecularly HLA-identical donor. All donor/recipient pairs were typed at the allelic level. Only donor/recipient unrelated pairs matched for both alleles were included. Diagnosis were AML (n=27), ALL (n=3), myelodysplastic syndrome (n=13), and myeloproliferative syndrome (n=15). Of the 32 males patients, 43% received graft from female donor. Medians age of recipients and donors at transplantation were 58 years and 47.1 years. Patients received either low-dose TBI (2Gy) (n=46) or Busulfan-based (n=12) conditioning regimen. Antithymoglobulin was given to 12 patients. As usual in RIC setting, Peripheral Blood Stem Cells was the main source of graft (65%), otherwise marrow graft (n=20). Results: with the median of follow-up of 27.2 months, 24 patients died including 9 from TRM. Relapse was recorded in 21 patients. Eighteen patients experienced acute GVHD (aGVHD) including 12 with II-IV grades and 7 with III-IV grades. Contrary to what we have previously reported in myeloablative allo-CST sittings, patients who underwent RIC and received graft from unrelated HLA-matched (10/10) donor, experienced worse outcomes compared to those transplanted with an HLA-identical sibling. Indeed, in multivariate analyses, donor type was the most important risk factors negatively influenced the overall survival and EFS [p=.01; HR=3.068; [95%CI: 1.312-7.174]) and (p=.050; HR=2.081; [95%CI: 1.001-4.347]), respectively.
This is the first study which compares results of sibling transplantation to HLA-allellically-matched unrelated transplantation. RIC allo-CST is still an attractive option especially for patients with high-risk malignancy.

P986

Successful engrafment after adult cord blood transplantation using a 4 Gy TBI reduced-intensity conditioning

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Objectives: Reduced-intensity Conditioning (RIC)-Unit Cord Blood Transplantation (UCBT) using Total Body Irradiation (TBI) has proved its efficacy for advanced hematological malignancies treatment.
The aim of this retrospective study was to analyze the feasibility and the efficacy of a 4 Gy TBI-based conditioning regimen before RIC UCBT in order to decrease the dosage of fludarabine, thereby limiting its neurotoxicity.
Methods: 10 patients with advanced hematological diseases (median age: 47 years; range: 18-60) underwent UCBT between 05- 2008 and 10- 2010. Preparative regimen comprised fludarabine 150 mg/m², cyclophosphamide 50 mg/kg, and 4 Gy TBI. They received one (n=2) or two (n=7) cord blood (CB) units, fully or partially matched (≥ 3 out of 6 HLA-antigen). Graft-versus-host disease (GVHD) prophylaxis comprised cyclosporine and mycophenolate mofetil.
Results: The median follow up is 18 months (range 2-30). Median infused total cell dose was 4,9 107 /kg (range 3,1- 7,8 107/kg). All the patients achieved primary neutrophil engraftment (ANC> 500/mm³) with a median day of engraftment to 32 days (range, 19–48 days). The platelet recovery (>20000/mm3) occurred at a median of 40 days (range 28-119). No secondary graft failure was observed. Median chimerism was evaluated in 7 patients and was 99,8% at 3 months (range 83-100%) and 100% in all the patients at 6 months. There was no transplantation related mortality (TRM) at 1 year, and 1 out of 6 evaluable patients died because of TRM at 18 months. 8 patients developed acute GVHD (n=9): grade ≤ II occurred in 7 patients and only 1 developed a grade III acute GVHD. Of the patients

who survived > 100 days (n=8), 3 developed chronic GVHD. Reactivation of CMV was documented in 5 patients and none of them developed CMV related disease. No fungal infection was observed and 1 patient developed a toxoplasmosis with a favorable evolution. No neurotoxicity was observed. Estimated 1-year overall survival (OS) and Event Free Survival (EFS) were 87,5%.
Conclusions: Although this study concern a small sample size and a short follow up, our results demonstrated the feasibility and the efficacy of a 4 Gy TBI–based conditioning regimen as an alternative to conventional RIC UCBT, for adult patients with advanced hematological diseases. It may provide sufficient immunosuppression, with a delayed hematopoietic recovery but without increasing risk of infection and 1-year TRM.

P987

Prognostic factors and outcome after reduced-intensity conditioning haematopoietic stem cell transplant: a single-centre experience

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Introduction: The efficacy of allogeneic haemopoietic stem cell transplantation (HSCT) is limited by concomitant toxicity. In the last decade, this has led to the development of less toxic, reduced intensity conditioning (RIC) protocols, whose therapeutic benefit is largely related to an associated immunity-mediated graft-versus-malignancy effect rather than by the cytotoxic treatment itself.
Aim: To analyze prognostic factors and outcome of RIC transplant in our center.
Patients and methods: We performed a retrospective study of 55 consecutive patients who underwent RIC-HSCT in our center between January 2001 and December 2009.
Results: Median age was 58 years (range 22-70), 29 males and 26 women. The median follow-up was 550 days range (22-3289). The distribution of underlying disease was: Acute leukaemia 17 patients (31%), non-hodgkin lymphoma 14 (27%), Hodgkin lymphoma 5 (8%) mulptiple myeloma 9 (16%), myelodisplastic syndrome 8 (15%) and other 2 patients (3%). The number of previous regimens (PR) before-transplant was 1 in 20 patients (36%) and >1 in 35 patients (64%). In 72 % of the cases the donor was HLA identical sibling. The distribution of acute graft versus host disease (aGVHD) was 23 patients (41%), with a median day of onset after transplant was +43 (range 11-151). As for the chronic graft versus host disease (cGVHD), 36 patients (65%), presented some sign, with median day of onset +183 (range 91-456), being a severe grade in 20% of the cases. The OS was 62% (34 patients). OS at +100 was 89% and at 1 year of 71%. The relapse rate (RR) was 11%. Transplant Related Mortality (TRM) was 33% (n=18), being on the +100 of 7% (n=4), and at one year of 22% (n=12). The main cause of death was infection: 13 patients (61%). In the univariant analysis for the survival development of aGVHD, absence of cGVHD, the number of PR before transplant and the unrelated donor were unfavourable factors ($p<0.05$), whereas in the multivariant analysis, the absence of cGVHD and the number of PR before transplant were the only factors that showed significance ($p<0.001$; OR:7.98 and $p<0.05$; OR:3.621) respectively.
Conclusion: The TRM in our series is similar to the previously described in literature and confirms a better survival in the patients who develop cGVHD. This confirms the close relationship between cGVHD and GVL and, therefore, the importance of the immune mechanism in the treatment of the disease.

P988

Allogeneic haematopoietic cell transplantation after reduced-intensity conditioning regimen in 19 children with malignant and non-malignant disorders

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RIC regimens prior to allo-SCT are a well-established conditioning in elderly and unfit adult patients. The development of RIC allo-SCT in pediatric patients has been slower than in the adult population because children generally tolerate more intensive myeloablative regimens. However, an increasing proportion of heavily pre-treated pediatric patients might benefit from a non intensive conditioning. Thus far, data regarding the efficacy of RIC approaches to treat pediatric patients is still limited, and their role in pediatric cancer has yet to be defined.
The aim of this single-centre retrospective study was to assess the outcome of 19 children (Median age: 12.1 (range, 2.6-18.1) years; gender: male/female 10/9) treated with RIC allo-SCT for different hematological malignancies (n=17; ALL: 6; AML: 4; JMML: 2; NHL: 1; MDS: 1; sAML: 1; biphenotypic leukemia: 1; CML: 1), bone marrow failure (n=1) and neuroblastoma (n=1). In this series, all children were ineligible for a conventional myeloablative conditioning regimen because of severe comorbidities (n=9), a previous auto or allo-SCT (n=7) or a history of extensive chemotherapy (n=3). At time of RIC allo-SCT, most of the patients were in complete remission (n=13; 68%), 2 in partial response and 4 in stable or progressive disease. All patients received a fludarabine-based RIC regimen before allo-SCT (Flu-i.v.Bu-ATG: 8; Flu-Cy-low dose TBI: 7; other combinations: 4). The allogeneic graft was obtained from a match-related donor in 5 cases, match-unrelated donor in 6 cases, and unrelated cord blood (UCB) cells in the remaining 8 cases (42%). The median infused number of CD34+ stem cells were 4.94x106 /kg for peripheral blood stem cells or bone marrow and 0.155x106/kg for UCB stem cells. Two patients who received UCB failed to engraft and the median time to ANC>500/μL was 18 days. With a median follow up of 25 (range, 12-120) months after allo-SCT, treatment related mortality incidence was 16% (n=3). The principal cause of death was relapse (n=6) which occurred at a median time of 112 (range, 29-406) days after RIC allo-SCT. Only 2 patients experienced grade 3-4 acute GVHD and one patient developed chronic GVHD. At 2 years, the Kaplan-Meier estimates of disease-free (DFS) and overall survival (OS) were 45% (95%CI, 25-67%) and 55% (95%CI, 33-75%).
In all, these data suggest that favorable outcomes can be achieved with RIC allo-SCT in pediatric patients who are ineligible for standard myeloablative conditioning.

P989

Feasibility of tandem auto/reduced-intensity T-repleted haplo-identical bone marrow allograft for relapsed/refractory Hodgkin's lymphoma

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Tandem auto/reduced-intensity allograft (auto-SCT/RIC-allo) is a feasible approach in poor prognosis Hodgkin lymphoma patients (HL). For patients without matched related or unrelated donor, haploidentical donor could be a viable donor. Post-transplantation cyclophosphamide (Cy) is effective to prevent graft versus host disease (GVHD) and graft rejection, using T-replete bone marrow.
To investigate feasibility of auto-SCT followed by RIC-haplo with high-dose post-transplantation Cy in HL patients.
5 HL patients were treated. Patients not in FDG-PET complete remission (CR) after 2 chemotherapy lines were included. No patients were relapsed after previous HDC. High dose chemotherapy (HDC) consisted of Melphalan 200 mg/m^2 in 4 patients, and BEAM in 1 patient. RIC-haplo conditioning consisted of

fludarabine (30 mg/m2 x 5 days), Cy (14.5 mg/kg/day x 2 days, and TBI (2 Gy). Unmanipulated bone marrow cells were infused (target MNC dose 4 x 108/kg of recipient). GVHD prophylaxis consisted of Cy 50 mg/mKg/day for 2 days (d +3 and +4), followed by tacrolimus and MMF (starting from d +5).
At time of auto-SCT, three patients had progressive disease (PD), one stable disease and one partial response (PR). After auto-SCT, 2 patients achieved CR and 3 patients PR. Median time between transplant procedures was 2.5 months (range 1-3). Sorror score was 0 for alla patients. Median number of MNC, CD34+ and CD3+ cells in the graft were 3.6 x 108/kg, 3.7 x 106/kg and 40 x 106/kg respectively. Median time to myeloid and platelet engraftment was 22 (17-32) and 31 (21-43) days, respectively. Median hospitalization time was 32 days (range 23-48). Between day 30 and 45 days after RIC-haplo, all patients achieved a full donor chimera. No graft failure occurred. After tandem procedure 5/5 patients obtained CR. After a median observation time of 200 days (37-524), 5/5 patients are alive, 4 in CR and 1 in PD. Two patients developed skin acute GVHD (one grade I and one grade II). No chronic GVHD was observed. Infectious complications were depicted below.
These results suggest that tandem auto-SCT/RIC-haplo with high-dose post-transplantation Cy is feasible and well tolerated, with no enhanced toxicity. These preliminary data should be confirmed in more consistent cohort of patients.
Viral infections
CMV 4/5
EBV 1/5
BK 1/5
Bacterial infections
Pneumonia 3/5
Gram negative sepsis 2/5
Gram negative cystitis 1/5

P990

An improved mouse model of haematopoietic stem cell transplantation for investigating reduced-intensity conditioning regimens

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Haematopoietic stem cell transplantation (HSCT) can be successfully used to treat leukaemia and some inherited genetic disorders. Allogeneic transplant rejection remains a considerable risk and the development of reduced intensity conditioning (RIC) regimens is of importance to the field. It is difficult to test clinically relevant RIC regimens in mouse models of HSCT, partly because transplant is much easier to achieve in mouse models than in humans and partly because some aspects of the immune response differ.
Our aims were therefore to develop a mouse model that exhibits allogeneic HSCT rejection following full myeloablation, and secondly to use this clinically relevant model to test novel RIC regimens. To this end we have performed allogeneic HSCT in recipient mice treated with fully myeloablative doses of the chemotherapy drug Busulfan, and transplanted with clinically relevant bone marrow doses. This closely mimics the clinical regimen used for patients receiving HSCT for Hurler syndrome in Manchester, differing only in the absence of cyclophosphamide or fludarabine treatment.
Autologous HSCT is always successful in C57BL/6 mice when using 125mg/kg Busulfan pre-transplant, and this dose is equivalent to lethal (10Gy) irradiation. We tested transplantation from the MHC mismatched CBA strain to C57BL/6 mice using these conditions and show that they are insufficient to allow graft acceptance, whilst rejection coincides with a significant recipient T cell response. Allogeneic HSCT is successful when 125mg/kg Busulfan is combined with non-depleting anti-CD4 and anti-CD8 monoclonal antibody (mAb) treatment, blocking signal 1 transduction. However, these mAbs do not allow significant reduction of the Busulfan dose, as their use in combination with 100mg/kg Busulfan leads to rejection in more than half the recipients, and with 75mg/kg Busulfan leads to rejection in all recipients.
Our robust mouse model of allogeneic HSCT has shown the conditions required for transplant acceptance and rejection. This model is now being used to assess the major factors limiting engraftment in RIC regimens, including the immune system, migration and homing, chemokine levels, the stem cell niche and cell dose. This model will enable us to determine the most important factors in clinical transplantation and inform novel clinical RIC regimens.

P991

Reduced-intensity conditioning allogeneic haematopoietic stem cell transplantation for low grade non Hodgkin's lymphoma: preliminary results at Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil

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Introduction: Advanced low grade non-Hodgkin Lymphomas (NHL) have many and varied treatment options. For patients with relapsed or refractory disease, outcome following only chemotherapy protocols remains poor. The standard Conditioning Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) might be an alternative for relapsed low grade NHL, but its high Transplant Related Mortality (TRM), varying from 24% to 54%, accounts for a lower overall survival in this group. Reduced Intensity Conditioning (RIC) HSCT has been shown to be a good alternative to myeloablative regimens, with lower TRM, Graft versus Lymphoma Effect, and appears to be associated with improved outcome, presenting a 3 years disease free survival and overall survival from 32% to 66% and from 32% to 73%, respectively, depending on factors such as disease stage before transplantation.
Patients and methods: Sixteen heavily pretreated patients with low grade NHL were submitted to RIC HSCT with matched related donors at the Bone Marrow Transplantation Unit at HCPA between the period of September 2002 to October 2009.
Results: The mean age was 43 years and 7 patients were male and 9 female. Fifty percent (8) of the patients had refractory disease pre-HSCT, 43.8% (7) were at third complete remission (CR) and 6.2% (1) at second CR. Median of CD34/kg infused was 10.7 x 10^6 and the median of days for engraftment was 14.53. Conditioning regimens were Fludarabine + Cyclophosphamide in 3 patients, Fludarabine + Melphalan in 7, Fludarabine + Cyclophosphamide + Rituximab in 2, Fludarabine + Cyclophosphamide + Melphalan in 1, Cyclophosphamide + Total Body Irradiation (TBI) in 1 and Fludarabine + Cyclophosphamide + TBI in 2. Acute Graft versus Host Disease (GVHD) occurred in 50%, with 37.5% of those were steroids resistant; and 62.5% of the patients evolved to chronic GVHD, with 80% extensive. Chimerism from 9 patients was analyzed, and 89% were complete. Mean overall survival was 2.95 years; 62.5% of patients were alive within 1 year and 40% within 3 years. TRM was 6.66%. Eight patients died, five of them were transplanted with refractory disease. Two patients died of relapse, 5 of infections and 1 of acute GVHD.
Conclusion: Low grade NHL patients treated at HCPA with RIC HSCT had an overall survival and TRM comparable to the observed at other centers, appearing as a good alternative for this heavily treated group with less toxicity than myeloablative regimens. KEYWORDS: Lymphoma, allogeneic HSCT, RIC.

P992

Allogeneic stem cell transplantation in elderly patients

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Historically, an age >55y was considered a contraindication for allogeneic stem-cell-transplantation (allo-SCT). In the recent years, several publications showed that the age of 60y is not a valid cutoff age for allo-SCT. The present report summarizes our cumulative experience in a cohort of 19 patients aged more than 65 (age 65-75, median 67y) with hematological malignancies, treated with allo-SCT. Pre-transplant risk factors included: previous SCT (auto – 2, allo – 2), coronary artery disease – 7, diabetes mellitus – 6, hypertension – 4, life-threatening infections – 4, chronic renal failure – 3, others – 3. Most patients (n=16) received a fludarabine and busulfan/busulfex based regimen and 13 patients received reduced intensity conditioning. Time to recovery of absolute neutrophil count>0.5x10^9/L was not different than younger patients with a range of 9-19d (median 14d). The time interval to platelet recovery>20x10^9/L was 9-188d (median 12d). Veno-occlusive disease occurred only in 6/19 procedures and subsided with conventional treatment. One year non-relapse mortality occurred in 8 of the 19 patients (42%). Non-fatal non-transplant related complications occurred in 6/19 (32%) procedures including: transient cardiac, renal and neurological events. Fourteen of the 19 patients were discharged to further out patient follow-up. One patient developed squamous cell carcinoma of the pharynx (primary risk factor was heavy smoking) that led to his death 62 months post transplant while in remission from refractory MDS/AML and without GVHD. The one year event-free survival is 40%. These results further strengthen our current approach which does not use chronological age as a barrier for allogeneic transplantation and the current transplantation selection criteria.

Stem cell research, Regenerative medicine and Non-haematopoietic stem cells

P993

Comparative microRNA expression profile among umbilical cord, bone marrow and apheresis progenitor cells

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Aim: In this study, we compared expression levels of miRNAs in progenitor stem cells (SC) derived from various sources to clarify how miRNAs regulate hematopoiesis and commitment.
Material: Five samples of Unit Cord Blood (CB) were obtained from Calabria Cord Blood Bank of the Reggio Calabria Hospital-Italy. Bone marrow blood (BM) was collected from 5 healthy volunteers. Four haematological peripheral cell apheresis (HPC-A) samples were obtained from healthy volunteers mobilized with G-CSF. To obtain CD34+ SC from each blood source we performed mononuclear cells separation following the immune magnetic selection. The purity of isolated cell lineages was >92%. MirNAs were extracted using Ambion® mirVana™ Kit. Equal amounts of miRNA from each SC sample were mixed to generate three different miRNA pools: CB, BM, HPC-A-SCs. TaqMan Low Density Arrays (Applied Biosystem) were used for expression profiling of 760 miRNAs in three SC pool. In experimental setting the CB-SC and BM-SC miRNA pools were our targets, while HPC-A-SC was considered as calibrator. Because it was assumed that HPC-A-SC expression value of each calibrator miRNA was 1 unit, miRNA targets were considered up-expressed if showing a value >1 or down-expressed if <1.
Results: We discriminated 3 blocks of miRNAs expression pattern. Block A included miRNAs with similar behaviour between CB and BM but different respect to HPC-A-SC. Inside this group was possible detected subset of miRNAs up or down expressed, table 1. Interestingly, three monocytopoiesis members (miR-17, miR-20a, and miR-106a) were abundantly expressed in CB / BM CD34+ cells. Others miRNAs involved in the doubling of B-lineage cell differentiation (miR146a, miR181a) were highly expressed in CB and BM-SC. In contrast, miR221, miR222, miR223 were down-expressed respect to CD34+HPC-A SC, this is expected because they are confined in B and myeloid cell lineages. Block B included miRNAs with highest expression in CB-SC, miR-142-3p and miR-15 could be representative for this pattern. They are known important regulators of hematopoiesis and in BM-SC and HPC-A-SC miR-142-3p and miR-15 were similarly expressed. Block C included few miRNAs with similar values between them such as miR145 and miR320
Conclusion: Our data underscore complexity of miRNA regulatory network and indicate that miRNAs modulates HSC status and commitment. We can hypothesize a hierarchy among adult stem cells with CB-SC on the top.

Table 1.
Subset of miRNAs representative for different signature between Cord- Bone Marrow and Apheresis Progenitor cells.

ID miRNA	Expression level CB-CD34+SC	Expression level BM-CD34+SC	Expression level HPC-A-CD34+SC	
hsa-miR-92 a	18.920.654	22.429.206	1	UP
hsa-miR-126	18.522.127	24.902.127	1	UP
hsa-miR-106a	15.045.664	40.830.193	1	UP
hsa-miR-146a	11.121.701	30.376.122	1	UP
hsa-miR-17	54.535.766	2.375.121	1	UP
hsa-miR-20 a	38.750.477	10.003.438	1	UP
hsa-miR-21	72.366.877	19.947.398	1	UP
hsa-miR-30 c	75.794.063	68.797.860	1	UP
hsa-miR-17	43.286.133	54.535.766	1	UP
hsa-miR-181	41.967.068	32.477.303	1	UP
hsa-miR-140-3p-	0.0674641	0.19684765	1	DOWN
hsa-miR-150	0.009702258	0.3275562	1	DOWN
hsa-miR-16	0.056780744	0.3220538	1	DOWN
hsa-miR-186	0.12777531	0.71086264	1	DOWN
hsa-miR-196b	0.011692111	0.48322517	1	DOWN
hsa-miR-199a-3p-	0.22490852	0.68144566	1	DOWN
hsa-miR-223	0.03743413	0.25724074	1	DOWN
hsa-miR-323-3p	0.08021362	0.48443925	1	DOWN
hsa-miR-342-3p-	0.021423223	0.30785188	1	DOWN
hsa-miR-345-	0.007013804	0.049641885	1	DOWN
hsa-miR-454	0.014074132	0.14815646	1	DOWN
hsa-let-7e-	0.0071759196	0.29066864	1	DOWN
hsa-miR-486-3p-	0.0070430483	0.008195366	1	DOWN
hsa-miR-222	0.063295536	0.1196035	1	DOWN
hsa-miR-221	0.598555	0.726587	1	DOWN
hsa-miR-19 a	0.05923922	0.060175383	1	DOWN
hsa-miR-191	0.08344786	0.08443979	1	DOWN
hsa-miR-195	0.31359777	0.4724732	1	DOWN
hsa-miR-484	0.037297342	0.043575403	1	DOWN
hsa-miR-24	0.5449951	0.6317381	1	DOWN
hsa-let-7b	0.0037235091	0.048563905	1	DOWN

P994

Placental-derived stem cells improve haematopoiesis and immune-tolerance after allogeneic haematopoietic stem cell transplantation in murine models

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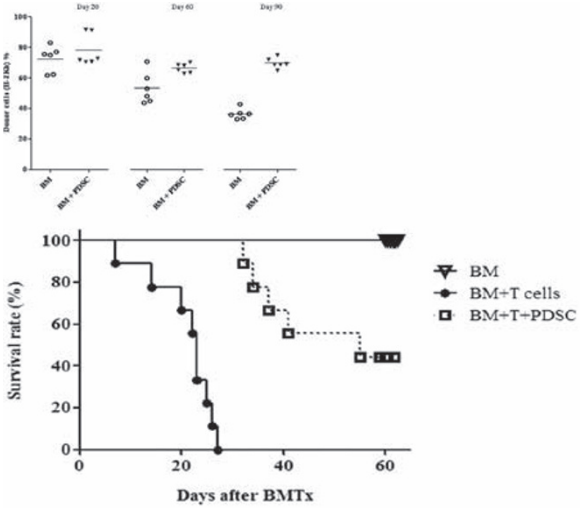
Introduction: Placental-derived stem cells (PDSCs), which are derived from deciduas basalis of placental, represent a new type of stem cells with multi-potent differentiation capacities. Because some phenotypic similarities exist between PDSCs and bone marrow-derived mesenchymal stem cells, we reasonably hypothesize that PDSCs could support hematopoiesis and induce immune-tolerance during allogeneic hematopoietic stem cell transplantation (allo-HSCT). This study is then aimed to test this hypothesis in in vitro culture and animal models.
Methods: Colony-forming unit (CFU) assay and one-way mixed lymphocyte assay are used for in vitro culture system to evaluation hematopoietic support and immune-tolerance induction of PDMCs. Mice allo-HSCT model is accomplished by infusing 1x10^4 c-kit+/sca-1+ hematopoietic stem cells (HSCs) from BALB/c H-2Kb donors into BALB/c H-2d recepients one day

after sublethal irradiation. If graft-versus-host disease(GVHD) is further needed, 2x10^6 donor CD3+ T-cells are co-infused with HSCs. Co-infusion of 2x10^6 phenotypically confirmed PDSCs will be done once if their effects are going to be demonstrated. Engraftment in tissues and blood is documented by analyzing the percentage of H-2Kb+ cells in flow cytometry.
Results: In CFU assay, PDSCs showed higher abilities to support hematopoiesis than mEF cells. In allo-HSCT model, PDSCs supported hematopoiesis with the evidences that co-infusion of PDSCs maintained higher levels of donor hematopoiesis than HSCs alone (figure 1). On the other way, PDSCs also could suppress Con-A or allo-antogen stimulated T-cell proliferation without causing T-cell apoptosis, and both cell-to-cell contact effects and soluble immune-modulatory factors contributed to the effect. In mice GVHD models, PDSCs co-infusion could significantly reduced the severity of GVHD and then rescue mice from death (figure 2). The number of CD3+ T-cells in blood was decreased, but donor cell engraftment in blood, marrow, thymus and spleen was not compromised. Delayed and decreased interferon-γ rising in blood was also noted.
Conclusion: PDSCs could demonstrate the abilities to support HSC engraftment and induce immune-tolerance after allo-HSCT in murine models. Human clinical trials are therefore reasonable for the next step. Furthermore, illustrating the possible mechanisms of action is also mandatory.



P995
Vascular regeneration in vivo is promoted by oxygen sensing mesenchymal stem and progenitor cells
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Vascular repair is a key mechanism during bone marrow regeneration post transplantation that requires a stringent interaction of somatic endothelial colony-forming progenitor cells (ECFC) with mesenchymal stem and progenitor cells (MSPC). Hypoxia is an established initiator of the revascularization machinery. Because ECFC in vivo, despite hypoxic stimulation, only form patent vessels in the presence of MSPC, we hypothesized that MSPC play a decisive role in oxygen sensing during vasculogenesis.
Adult human ECFC were isolated from blood and MSPC from bone marrow aspirates and expanded under humanized culture conditions. Throughout this study we designated the oxygen level in the venous environment as euoxia (5%O2). Levels below euoxia are defined as hypoxia (1%O2). Air-oxygen commonly used in standard laboratory practice is termed hyperoxia (21%O2). Progenitor cell phenotype, long-term proliferation, molecular cellular response, wound repair as well as migratory and vasculogenic functions were monitored under these oxygen levels. ECFC and MSPC interaction in vivo and the influence of MSPC protein synthesis were studied in immune-deficient NSG mice after subcutaneous transplantation. Immune histochemistry and TUNEL assays were performed on plugs in the time course after transplantation.
In vitro ECFC and MSPC proliferation was lower under hypoxic than euoxic, as compared to hyperoxic conditions despite unchanged progenitor colony number. ECFC vascular wound repair and vascular-like network formation in vitro improved with escalating oxygen supply. ECFC stabilize hypoxia-inducing factor-1α (HIF-1α) only under hypoxia, while MSPC stabilize HIF-1α under hypoxic as well as euoxic conditions. In a mouse model, ECFC underwent apoptosis after 24h and attracted mouse leucocytes. In vivo co-cultured ECFC and MSPC formed perfused human vessels 7 days after transplantation. Perivascular cells, but not ECFC, were positive for HIF-1α in vivo. Inhibition of MSPC protein synthesis prior to co-implantation blocked vessel formation.
These data indicate that hypoxic ECFC alone are not able to function sufficiently in vitro and form patent vessels in vivo. MSPC react to the low oxygen environment and promote ECFC to form vessels at least in part by rescuing ECFC from hypoxia-induced apoptosis. This data suggests that in addition to their established role regulating hematopoiesis, MSPC may also be crucial during bone marrow vascular regeneration.

P996
Unexpected somatic restriction of mesenchymal stem and progenitor cells after transplantation in vivo
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Multipotent mesenchymal stem/progenitor cells (MSPC) are considered to represent a universal skeletal progenitor capable to regenerate bone and the hematopoietic stem cell niche. Osteo-, chondro- and adipogenic differentiation in vitro are generally considered to predict multipotentiality making them attractive candidates for clinical transplantation. This study was performed to define the potency MSPC in vivo.
Bone-marrow (BM), white adipose tissue (WAT), umbilical cord (UC) MSPC and skin fibroblasts were isolated and propagated under humanized conditions using pooled human platelet lysate (pHPL). Differentiation was tested in vitro and in vivo after transplantation and long term lineage stability was determined for up to 12 weeks in NSG mice. In vivo osteogenesis was monitored by near infrared imaging and micro-CT. Lineage commitment in vivo was confirmed by histology.
Adipogenic differentiation and subsequent Oil Red O-reactive lipid storage in vitro was inducible in all cell types. All MSPC as well as fibroblasts showed evidence of osteogenic commitment resulting in calcification as indicated by Alizarin Red staining with a clear quantitative graduation of WAT>BM>Fibroblasts>UC confirmed by calcium analysis. Surprisingly only BM-MSPC formed human bone, cartilage and adipose tissue in all transplants analyzed. Human origin of long-term stable bone was confirmed by immune histochemistry. Furthermore only BM-MSPC showed full functionality forming a hematopoietic marrow niche and attracting complete mouse hematopoiesis. Chondrogenic differentiation with subsequent mucopolysaccharide deposition was also restricted to BM-MSPC as evaluated by Alcian Blue, Toluidine Blue and Safranin O staining. MSPC derived from WAT could only differentiate into adipose tissue whereas cells generated from all other sources just formed fibrous tissue in vivo.
Using an ectopic subcutaneous transplant model in NSG mice, we found that only human BM-MSPC can differentiate into bone and a marrow niche that subsequently attracts hematopoiesis in vivo. Despite positivity in osteogenic differentiation assays and supposed reactivity in chondrogenesis assays in

vitro, WAT- and UC-MSPC could neither form bone nor cartilage in vivo. We conclude that in vitro differentiation is too permissive to predict the tissue-regenerating potential of MSPC in vivo. Our data indicate that BM may represent the only MSPC source capable of clinical bone regeneration and hematopoiesis support in vivo.

P997

Stem cell therapy to counteract the loss of regenerative capacity of colon after treatment of colorectal cancer: pre-clinical study

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Radiotherapy is successfully used to cure prostate, uterus, and rectum cancers. Although, it is reported that the patients receiving radiation therapy shows early or late tissue reactions of graded severity as radiotherapy affects not only the targeted tumor cells but also the surrounding healthy tissues. Unfortunately, the medical management of those patients who experienced adverse effects remains actually insufficient. Our group investigates the therapeutic benefit of cell therapy to counteract the loss of regenerative capacity of the healthy tissue after radiotherapy. Moreover, side effects of stem cell injection are carefully studied for further application in patients. We have investigated whether MSC may promote colon and rectum regeneration after fractionated irradiation without favouring colorectal cancer development. We retain to use Mesenchymal Stem Cells because they have a successfully use in clinical trials. Our group previously showed that MSC treatment increases and accelerates the recovery of the intestine after radiation exposure. To study the influence of MSC on tumours, MSC are injected intravenously following cancerogenesis induction by intra-rectal injection of the alkylating agent N-Methyl-N'-Nitro-N-Nitrosoguanidine (MNNG). Our results validate the colorectal cancerogenesis model in Sprague-Dawley rats. Ten weeks after MNNG treatment ACF appear that express the tumoral marker M1/MUC5AC in the distal colon. From 32 to 52 weeks post-MNNG treatment adenomas and adeno-carcinomas are revealed. The formation of Aberrant Crypt Foci (ACF) and tumours from ten to fifty-two weeks were compared between MNNG-treated rats with and without MSC infusion. Results clearly demonstrate that MSC therapy not promotes colorectal carcinogenesis in rat. We are now studying whether MSC prevent loss of colon and rectum function after radiotherapy. Our preclinical study of cell therapy to counteract the loss of regenerative capacity of colon and rectum after treatment of colorectal cancer will give the rational for clinical treatment.

P998

Human platelet-derived factors maintain a bone and marrow niche-competent function of mesenchymal stem/progenitor cells

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Mesenchymal Stem/Progenitor cells (MSPC) are thought to represent a universal skeletal progenitor capable of recreating the skeleton including the entire hematopoietic niche for continuous blood formation. Consequently clinical trials have been initiated testing the bone regeneration and hematopoietic support capacity of MSPC transplants. This study was initiated to select appropriate strategies to propagate MSPC for therapeutic application.
Bone marrow-derived MSPC were expanded following standard protocols comparing fetal bovine serum as a supplement (MSPC/FBS) with humanized culture conditions under the aegis of pooled human platelet lysate (pHPL). Expanded MSPC were inoculated in a non-mineral, collagen/laminin- matrix and transplanted in immune deficient NSG mice. Bone formation was monitored non-invasively by osteosensitive near infrared imaging and micro-CT and confirmed by histology. We found that MSPC/pHPL from 9/9 donors formed bone in vivo compared to only 2/9 MSPC/FBS. Extensive histo-morphological analysis in the time course of bone formation indicated that human platelet-derived factors promote endochondral ossification through a G-protein-coupled receptor (GPCR)-α mediated mechanism, as pre-treatment of MSPC with Cholera Toxin could efficiently hamper bone development in vitro and in vivo. Interestingly, most ectopic ossicles generated by MSPC expanded with pHPL became colonised by mouse bone marrow, indicating that platelet-derived factors maintain full functionality of MSPC including the capacity to reconstitute a functional bone-marrow niche. Human CXCL12+ cells were observed in situ in these ossicles admixed with a complete (mouse) hematopoiesis including all major blood lineages, suggesting that MSPC could also differentiate in vivo into CXCL12-abundant reticular (CAR) cells. This hypothesis was further supported by the observation that human MSPC could be re-isolated and re-expanded from fully differentiated bone-marrow infiltrated ossicles, suggesting that appropriate human platelet-derived factors may guide MSPC to retain their stem cell potential in the ectopic bone microenvironment.
We conclude that appropriate culture conditions combined with reliable (in vivo) potency assays may help to select optimal conditions to propagate MSPC for clinical application. Results of this study indicate that pHPL may help to increase the efficiency of MSPC transplants for bone and marrow regeneration.

P999

Mesenchymal stem cell for multiple sclerosis: a therapeutic breakthrough

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Purpose: Mesenchymal stromal stem cells (MSC) are known to down-regulate anti-self reactivity and have the potential to migrate to and to regenerate damaged tissues. Our goal was to explore the potential clinical benefits of enriched autologous bone marrow derived MSC in patients with multiple sclerosis (MS).
Patients & methods: A total of 58 MS patients (26-70 years old; 26 males+32 females), diagnosed 1-27 years prior to our treatment, with pre-treatment EDSS ranging from 1-8.5 were treated in our center during 2008-10. 43 relapsing remitting (RRMS) of which 19 developed secondary progressive (SPMS) and 15 patients had primary progressive (PPMS). Passage 0 MSCs were enriched in clean rooms by culturing of bone marrow aspirate and administered intrathecally ± intravenously.
Results: Other than mild fever and self-limited headache no patient experienced severe side effects. With an observation period <19 months, 41 out of 58 patients (RRMS = 17, SPMS = 15, PPMS = 9) reported subjective improvement starting at >3 weeks after MSC injection; some with significantly objective improvement of EDSS. 10 patients did not report any benefit and 2 of them feel they deteriorated. 7 patients are lost for follow-up evaluation. Evaluation of all patients at +3, +6 and +9 months is pending.
Conclusions: Autologous MSC may represent an innovative, safe and potentially beneficial treatment for MS patients and should be further investigated prospectively also at early stages of the disease.

P1000

Oxygen tension is a critical player in the haematopoietic stem cell microenvironment in vitro

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Background: In the bone marrow, hematopoietic stem cells (HSC) are in close proximity to mesenchymal stromal cells (MSC) and osteoblasts forming special niches. This microenvironment can be mimicked with co-culture assays in vitro. Recently, we described three spatial compartments with different characteristics in a HSC/MSC co-culture model. In this study, we investigated the impact of oxygen tension on HSC behavior in these three compartments.
Design and methods: Oxygen tension in the co-culture was evaluated by pimonidazole staining. The HSC distribution was studied according to cell numbers in the three compartments. Hypoxic co-culture was compared with normoxic co-culture regarding the characteristics of HSC subsets (i.e. immunophenotype, cell division, and migration) and cytokine secretion by MSC (i.e. VEGF-A and SDF-1).
Results: In normoxic co-cultures a hypoxic area could be detected beneath the MSC layer where a reservoir for more primitive HSC in vitro is located. In hypoxic co-cultures, the HSC adhesion to the MSC layer was diminished, whereas the cell migration beneath MSC was increased. The molecular mechanisms behind these effects include a higher VEGF-A secretion by MSC under hypoxic conditions since silencing of the VEGF-A expression enhanced the cell adhesion to the MSC surface. Moreover, hypoxic HSC express a significantly higher level of LFA-1 mediating the migration beneath the MSC layer. The process seems to be negatively modulated by the SDF-1 expression of MSC since SDF-1 level was down-regulated in a time-dependent manner under hypoxic conditions. Both VEGF-A and SDF-1 expressions were partly regulated through the HIF signaling pathway. The phenotype of HSC was maintained in all three compartments of hypoxic co-culture over time in comparison to the normoxic co-culture. An accumulation of more primitive HSC was still localized beneath the MSC layer.
Conclusions: We demonstrate for the first time that hypoxia caused significant modifications of both HSC and MSC in the co-culture confirming that oxygen tension is a critical player in the HSC niche in vitro.

P1001

CD133+ pluripotent stem cells for the treatment of chronic liver failure

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We showed that the administration of G-CSF to patients with liver cirrhosis is safe and allows the mobilization and collection of BM-derived stem cells (SCs) at the dose of 15 ug/kg/day. We also demonstrated that cirrhotic mice transplanted with mobilized CD133+ SCs appear to have less fibrotic septa and improved liver function than control mice, suggesting the potential therapeutic role of SCs for the treatment of liver fibrosis. Thus, we started a phase I clinical study with the main objective of evaluating the feasibility and the safety of the purification and intrahepatic reinfusion of increasing numbers of autologous G-CSF-mobilized CD133+ SCs in patients with end-stage liver disease. For this purpose, G-CSF is administered subcutaneosly (sc) from day 1 until the completion of SC collection. PB mononuclear cells obtained from mobilized standard-volume leukapheresis are incubated with Macs colloidal superparamagnetic CD133 microbeads and positive selection of CD133+ SCs is performed under GMP conditions. Cryopreserved, highly purified, autologous CD133+ SCs are then re-infused through the hepatic artery by transfemoral or transbranchial arteriography. CD133+ cells are administered to patients starting from $5x10^4$/Kg patient's body weight and increased every 3 patients. The planned maximum infused cell dose is $1x10^6$/kg. G-CSF at 5 µg/Kg/day is administered for 3 days after the reinfusion of SCs for their expansion and to induce a selective proliferative advantage of reinfused cells in vivo. Biological assays (flow cytometry of circulating SCs, clonogenic assay of hematopoietic and endothelial progenitors, serum cytokine concentration, molecular analysis of SCs) are performed during the mobilization and re-infusion phases together with the phenotypic characterization of the isolated CD133+ SCs. Up to date, five cirrhotic patients (HCV+=3; Alchool =2) have been successfully mobilized with G-CSF and highly purified autologous CD133+ SCs have been re-infused in 4 patients. The procedure appears to be safe and no side effects were recorded while we observed the decrease of bilirubin value in the first 3 evaluable patients. Biological studies indicate that:

1) circulating hematopoietic and endothelial progenitors are increased after G-CSF treatment;
2) isolated CD133+ cells show various expression of hematopoietic and endothelial markers;
3) re-infusion of CD133+ cells does significantly modify the circulating stem cell compartment.

P1002

Ex vivo expanded bone marrow CD34+ for acute myocardial infarction treatment: in vitro and in vivo studies

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Cell-based therapy is a promising option for the treatment of acute myocardial infarction (AMI). Among the issues that still need to be addressed, such as the quality and quantity of the hematopoietic stem cells to be used along with the appropriate time of administration, the availability of adequate quantity of cells was overcome by a previously established protocol that allows the expansion of hematopoietic CD34+ cells isolated from neonatal and adult hematopoietic tissues. As next aim of our in vitro and in vivo study, the feasibility and the safety of Bone Marrow expanded CD34+ cells (BMeCD34+) versus Bone Marrow basal CD34+ cells (BMbCD34+) as cells suitable for Cell Therapy Protocol (CTP) in the treatment of experimental AMI was addressed. We investigated possible biological mechanism contributing to cardiac protection. We evaluated gene profile, differentiation potential towards endothelial lineage, tissue regeneration and cytokine release. Gene profile analyses revealed that, during in vitro culturing, there is an upregulation of endothelial genes expression. Moreover, BMeCD34+ cells generated a CD14+ subpopulation that efficiently differentiated into VE-Cadherin expressing cells. By using an in vivo model of coronary artery ligation (CAL), we observed a functional recovery only in mice sequentially treated with BM bCD34+ cells and BMeCD34+ cells (BMbeCD34+) injected respectively 4 hours and 7 days after having performed CAL. Thus, our data suggest that combining basal and expanded BM-derived CD34+ cells in a specific temporal pattern of administration might represent a promising strategy for a successful cell-based therapy.

P1003

Use of bone marrow derived mesenchymal stem cells for muscle regeneration in a model of lower oesophageal sphincter damage

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Background: The incompetence of lower esophageal sphincter (LES) and the following exposition of the esophageal mucosa to the stomach acid content lead to a chronic disease called gastroesophageal reflux disease. In the last few decades several studies proposed the use of bone marrow mesenchymal stem cells (BM-MSC) in regenerative medicine for the treatment of urinary and anal incontinence. The aim of this study is to evaluate the therapeutic effect of a BM-MSC injection into heat-damaged LES of rats.
Methods: 24 inbred Wistar Furth rats were divided into three groups. Group A underwent a sham operation and saline injection. Group B had a sphincter myotomy plus saline injections. The study group C underwent a myotomy followed by intrasphincteric injections of syngeneic BM-MSC expanded in vitro. At day 30, histological and morphometric analysis and in vitro contractility experiments were performed.
Results: A significant ($p<0.05$) decrease of muscular tissue was observed at the site of myotomy in Group B. MSC injection (Group C) improved contractility of sphincter strips compared with Group B. Histological examination demonstrated new muscle fibres whilst morphometric analysis revealed a significantly ($p<0.05$) greater muscular area fraction than in Group B.
Conclusions: In our experimental model, BM-derived MSC injection improved muscle regeneration and increased contractile function of LES. Therefore MSC may represent an attractive tool for treating LES incontinence. Further investigations using GFP-MSC are under way and will increase knowledge on underlying mechanisms involved in MSC-based muscle regeneration in sphincter repair.

P1004

Hypoxic bone marrow stromal cells create a pro-survival niche for breast cancer stem cells

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Recent literature indicates that cancer stem cells are responsible for the dissemination of breast carcinoma tissues at distant sites. In the bone marrow, an exquisite site of early breast cancer dissemination, the long-term survival of hematopoietic stem cells is maintained in sub-endosteal stromal niches. The environment where such structures are harboured is characterized by an extremely low oxygen tension (about 1% pO2). We here assessed the role of such an hypoxic environment on breast cancer stem cells survival. Human breast cancer stem cells were expanded in vitro as multicellular spheroids, called mammospheres, and were assessed for self-renewal capability upon exposure to hypoxia (1%pO2) or to supernatants of hypoxia exposed human bone marrow stromal cells. We show that human mammospheres cultured in presence of hypoxia disclose an enhanced self-renewal potential. This increase is several fold augmented by the administration of hypoxic bone marrow stromal cells supernatants to mammospheres. Exposure of stromal cells to 1%pO2 up-regulates Vascular Endothelial Growth Factor and increases Vascular Endothelial Growth Factor receptor in mammospheres. These findings support the notion that the bone marrow hypoxic niche environment, that physiologically sustains normal stem cell survival, may sustain breast cancer stem cells survival.

P1005

Expansion and functional characterization of mesenchymal stem cells derived from patients affected by Shwachman-Diamond syndrome

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Shwachman-Diamond Syndrome (SDS) is an inherited marrow failure disorder characterized by varying cytopenias, pancreatic dysfunction, and metaphyseal dysostosis. Neutropenia plays a crucial role in the occurrence of recurrent and severe infectious complications representing one of the major causes of death in SDS patients. The aim of our study is to better comprehend the marrow dysfunction occurring in SDS patients, by analyzing the functional properties of bone marrow (BM)-derived mesenchymal stem cells (MSCs). After informed consent, BM cells obtained from 20 SDS patients were plated in sterile tissue culture flasks. At the third passage of the culture, cells were tested for the expression of specific surface markers, their ability to differentiate into mesengenic lineages, their capability to abrogate T cell proliferation and their capacity to prevent neutrophil apoptosis. MSCs derived from SDS patients (SDS-MSCs) displayed typical fibroblastoid morphology; they were consistently devoid of contaminating hematopoietic cells, being negative for CD34, CD45, HLA-DR, CD11b, CD19, and CD14, but expressed common MSC markers including CD90, CD73, CD105 and HLA-ABC. Similarly to MSCs obtained from healthy donors (HD-MSCs), these cells were able to differentiate into adipocytes and osteoblasts. In addition, SDS-MSCs drastically decreased the mitogen-induced lymphocyte proliferation, in a dose-dependent manner. We also cultured neutrophils obtained from HD in presence or absence of MSCs at different time points. We demonstrated that SDS-MSCs were comparable to HD-MSCs in supporting the viability of neutrophils. Importantly, SDS-MSC were able to produce high amount of IL-6 (mean= 2658 pg/ml, range= 2086-3229 pg/ml), a crucial cytokine involved in the protection of neutrophils from apoptosis.
In conclusion, we successfully isolated and characterized MSCs from SDS patients. These cells did not show any significant differences from HD-MSCs. Further studies are needed to better comprehend the functional and molecular features of SDS-MSCs, which are potentially involved in the hematological abnormalities typical of SDS patients.

P1006

Imatinib mesylate versus allogeneic haematopoietic stem cell transplantation for patients with chronic myelogenous leukaemia in accelerated phase: a single-centre experience in China after a nine-year follow-up

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The relative merits of allogeneic hematopoietic stem cell transplantation (allo-HSCT) and imatinib for chronic myelogenous leukemia (CML) in accelerated phase (AP) have not previously been evaluated. This cohort study was designed to compare the outcomes of imatinib (n=87) versus allo-HSCT (n=45) for AP CML. A multivariate analysis of the total population revealed that CML duration > 12 months, hemoglobin < 100 g/L and peripheral blood blasts > 5% were independent adverse prognostic factors for both overall survival (OS) and progression-free survival (PFS). Neither imatinib nor allo-HSCT influenced the outcomes in low-risk (no factor) patients, with six-year event-free survival (EFS), OS and PFS rates of more than 80.0%. Intermediate-risk (any factor) patients showed no difference in EFS and OS, but six-year PFS rates were 55.7% vs 92.9% (P=0.047) with imatinib versus allo-HSCT. Among high-

risk (at least two factors) patients, imatinib was by far inferior to allo-HSCT, with five-year EFS, OS and PFS rates of 9.3% vs 66.7% (P=0.034), 17.7% vs 100% (P=0.008) and 18.8% vs 100% (P=0.006), respectively. We conclude that allo-HSCT, compared to imatinib, confers significant survival advantages for high- and intermediate-risk patients with AP CML; however, the outcomes of the two therapies are equally good in low-risk patients.

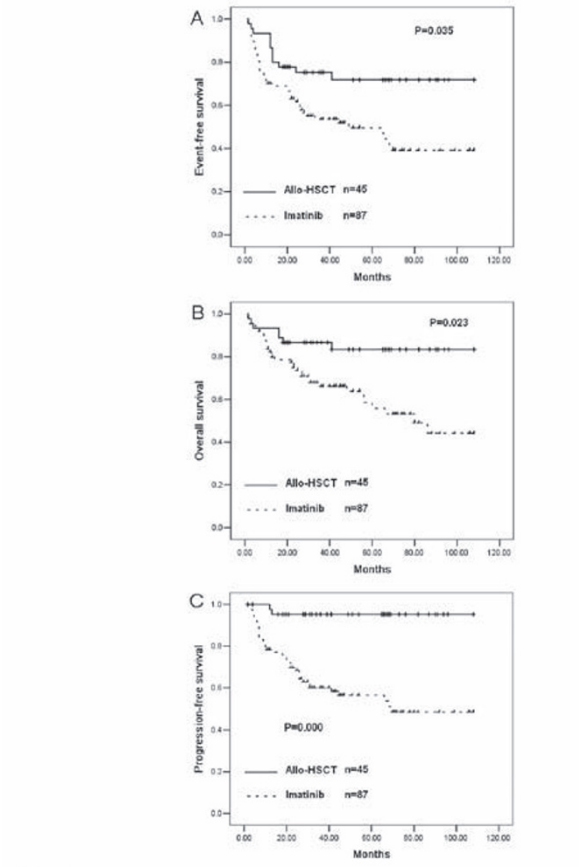
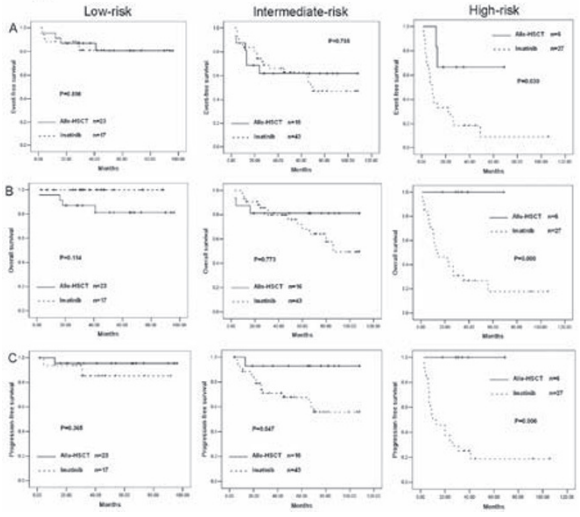


Figure 2



P1007

Platelet lysate as an alternative source for mesenchymal stem cells in vitro expansion

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Background: Due to existent perspectives of clinical usage of bone marrow mesenchymal stem cells (MSC) population most actual is development of their isolation, expansion and infusion methods. However at the present in the most protocols for MSC expansion the culture mediums with addition of fetal calf serum (FCS) are used which is a potential source of xenogenic antigens as well infection contamination. The human platelet lysate (HPL) from platelet enriched plasma could be considered as an alternative to FCS.

Objectives: The aim of the study was development of techniques of HPL extraction, evaluation its biological activity and possible use as an alternative to FCS.

Methods: Thirteen bone marrow samples from healthy donors were under study. Three different types of medium: 1) DMEM (Gibco) with fetal cow serum 15% FCS 2) Serum-Free Medium (Stem-Cell Technologies) 3) DMEM + 10% HPL. We investigated the proliferative activity of MSCs in primary culture (number of CFU-F colonies) and during long-term cultivation. MSC immune marker profiling was carried out by analysis of CD45+, CD14+, HLA1+/CD14-, CD45+/CD34+, CD34+/HLA DR-/CD45-, CD34+/HLA DR-/CD45+, CD34+/HLA DR- cells. Gene expression profile of the cells, yielded from the 3rd passage, was investigated by RT-PCR using a SYBR GREEN detection technique. Expression of several genes was analysed: NGF, SREBF, MPL, NGF, THPO, osteo, collagen, NTF3, ITGA 7, BGP, TNFR, WISP, BDNF. Osteogenic and adipogenic differentiation ability was tested. Hematopoiesis support function was tested by means of agar drop-liquid culture.

Results: In assessing of MSC's colony formation in primary culture the number of colony-forming units of fibroblasts (CFU-f) was higher when HPL was used in comparison with FCS. During long-term cultivation was no differences in MSC's proliferating capacity observed. The lower number of CD14+ cells and reduced level of NGF, osteopontin, ITGA7, NTF3 genes expression was identified when HPL was used. The ability of MSC's osteogenic differentiation was higher in HPL-supplemented medium. Testing for hematopoetic support functions did not show significant differences within 3 medium types.

Conclusion: During expansion of MSC's using HPL their differentiation ability was changed with accompanied gene expression profile changes and with comparable to FCS proliferation ability.

P1008

Flow cytometry of murine bone marrow: long-term repopulating activity should be attributed to lineage low, not to lineage negative subset

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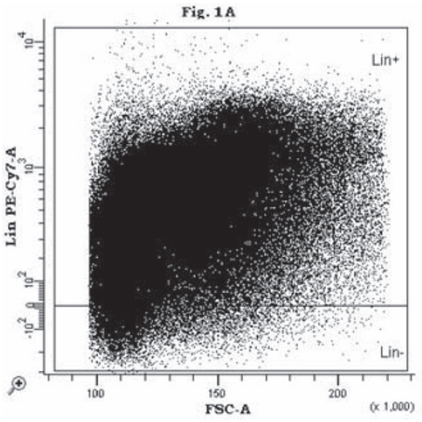
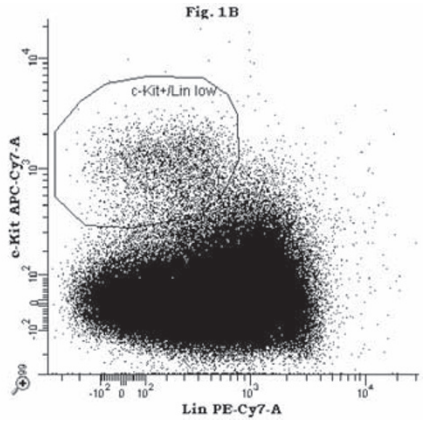
Objective: Murine hematopoietic stem cells (HSC) are assumed to be in lineage negative,

c-Kit positive, Sca-1 positive (LSK) subset of bone marrow (BM). Magnetic depletion of lineage positive (Lin+) cells is routinely used for enrichment purpose. We found that Lin+ fraction contains a lot of HSC after magnetic separation. Therefore we tried to characterize Lin+ and Lin- subsets in terms of presence of long-term repopulating activity (LTRA).

Methods: Different cell subpopulations were isolated by flow cytometric analysis and sorting (FACS, purity >95%) from either BM Lin+ or Lin- subsets of Ly-5.1 mice. "Lin vs FSC" one-fluorescence parameter dot plot was used for drawing Lin+ or Lin- regions, and about 5% of BM events were defined as Lin- (Fig 1A). Sorted subpopulations (equal fractions of femurs)

[P1008]





were transplanted into 6 Gy irradiated Ly-5.2 mice. Chimerism in recipients' peripheral blood was followed up to 4 months posttransplantation to analyze LTRA of donor-derived cells.
Results: First, we determined that LTRA is present in Lin+ subset of murine BM.
The engraftment of transplanted Ly-5.1 Lin+ cells was stable in multiple lineages up to at least four months. The LTRA level of Lin+ subset was very similar to LTRA level determined in Lin- subset. The LTRA in Lin+ subset was strongly associated with Side Population (SP) and c-Kit+ phenotype. We have then used the "c-Kit vs Lin" two-fluorescence parameters dot plot gating to sort c-Kit+/Lin low population (Fig. 1B) and transplant it to congenic recipients. Almost all LTRA was found inside this population and only very low chimerism (less then 1%) was achieved after transplanting of remaining bone marrow cells. Because new sorting gate using Lin low region partially overlaps
Lin+ region of previous gating, we conclude that our findings of LTRA in Lin+ subset were confusing due to inclusion of HSC in c-Kit+/Lin low gate into Lin+ subset.
Conclusion: To avoid confusing results, "c-Kit vs Lin" two-fluorescence parameters dot plot must be used to precise LTRA region designation. Attribution LTRA to "lineage-negative" subset in murine BM is misleading. Instead, attribution LTRA to "lineage low" subset should be used. Also, because Lin+ contain notable number of
c-Kit positive events, notable total loss of HSC after magnetic Lin+ depletion may be predicted.

P1009

Chondral lesions treatment by using bone marrow cells and hyaluronic acid scaffold

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With the aim to regenerate the cartilage of the knee, two patients affected by III-IV grade chondral lesions were treated locally by combining the use of a hyaluronic acid (HA) scaffold with autologous bone marrow (BM) buffy-coat and platelet enriched plasma (PRP).
Both the patients, 1 male age 30 years, 1 female age 38 years underwent knee arthroscopy. Intra-operatively 60 ml of BM were aspirated by the posterior iliac crest using heparin-coated syringes and concentrated by the SmartPrep2 Centrifuge System (Harvest Technologies GmbH) to a final volume of 7 ml. A HA membrane, used as scaffold, was embebbed with 5 ml BM concentrate (4-fold enriched with respect to mesenchymal stem cell-MSC recovery at P2 compared to starting BM) additioned with autologous PRP plus thrombin (1+1 ml) and 1 ml of Ca-Gluconate. The membrane was applied locally to completely cover the area of cartilage lesion. Two ml of fresh BM and 2 ml of residual BM concentrate were sedimented on Fycoll-Hypaque gradient and $5x10^6$ mononuclear cells (MNC) were cultured in 5 ml of LSF- Mesenchymal Medium (LI-StarFish) additioned with 10% of platelet lysate (PL). After 48 hrs the culture supernatant was removed and the adherent cells expanded to evaluate ex vivo growth and selection of MSC. Phenotypic analysis was assessed on MNC at the beginning and following expansion by using FacsCalibur and the monoclonal antibody recognizing CD45, CD90, CD105, CD71, CD73, CD166, CD34.
The MSC identified as CD45-, CD34-, CD105+, CD90+,CD73+, CD71+, CD166+ were very low represented (< 0.05%) both in fresh and concentrated BM. Within 3 weeks, the adherent cells from BM of both the patients reached >80% confluence and subsequently expanded under the appropriate culture conditions. Three passages including trypsin treatment, cell count and dilution for expansion were performed within 7 weeks before stopping the culture. The phenotypic analysis of the cells at the end of the expansion showed a cell population of > 95% MSC.
The follow-up at 5 months (patient 1) and 3 months (patient 2) showed in both subjects a significant reduction of the cartilage lesions, as evaluated by magnetic resonance imaging, and an improvement of the knee function
The use of BM as a source of MSC seems to be a feasible option for the treatment of focal lesions of the articular cartilage. The evaluation of this cellular approach in a pilot, dose-escalation study is now in progress at our site.

P1010

Cytokine-producing function of adipose- and bone marrow-derived mesenchymal stromal cells

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Despite intensive studies, we are still far from the conclusion that mesenchymal stem cells (MSCs) from different tissues are the same, and there is a possibility that various MSCs may have some features depending on the sources. So we have compared secretory activity of MSCs derived from bone marrow (BM) and adipose tissue (AT) of 11 healthy donors. MSCs were obtained by a standard procedure of adherent cell culturing in α-MEM with 20% FCS and passed at least 2-3 times before investigation. Cytokine, chemokine, and growth factor measurements were studied using the Bio-Plex Protein Array System (Bio-Rad, USA) and ELISA.
AT-MSCs in compare with BM-MSCs displayed more pronounced proinflammatory and immunoregulatory "phenotype" due to higher spontaneous production of IL-13, IL-17, IFN γ, IL-2 and IL-1b. In addition, AT-MSCs more efficiently secreted cytokines which are known to stimulate hematopoiesis

(eg, GM-CSF and erythropoietin). In contrast to BM-MSCs, AT-MSCs had lower sensitivity to LPS stimulation.
At the same time MSCs regardless of the source were comparable by levels of CXC- (IL-8) and CC chemokines (MCP-1, MIP-1b). Moreover, AT-MSCs and BM-MSCs were similar in the production of matrix metalloproteinase 9 (MMP-9) and its inhibitor, TIMP-1, that play an important role in morphogenetic and reparative processes being involved in adhesion, homing, proliferation and apoptosis of cells.
Thus, in compare with BM-MSCs, AT-MSCs possess a higher potential to support hematopoiesis (via production of G-CSF, GM-CSF and erythropoietin). At the same time, AT-MSCs display comparative capacity with BM-MSCs to stimulate neovasculo/angiogenesis (via production of VEGF, FGFb), neuroregeneration (via production of IGF-1, FGFb and IL-6), to regulate glucose metabolism (via production of IGF-1) and remodeling of extracellular matrix (via production of MMP-9 and TIMP-1)

P1011
Chemotherapy-induced damage to mesenchymal stem cells: an in vitro model
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Mesenchymal Stem Cells/Stromal Cells (MSC) form the bone marrow microenvironment and are essential in supporting haemopoiesis. Following stem cell transplantation (SCT), whilst haemopoietic cells are replaced, MSC remain of recipient origin. However, chemotherapeutic treatment has previously been shown to damage MSC, administered prior to SCT. If damage is severe it may be implicated in lack of engraftment following some SCT, a major cause of mortality, occurring in 10% of allogeneic peripheral blood transplants. To elucidate this damage, a physiologically relevant in-vitro model is needed as many chemotherapeutic agents are extensively metabolised by hepatic cytochrome P450 enzymes. Without adequate metabolism, overestimation of active drug damage can occur, whereas damage from prodrugs can be grossly underestimated due to lack of active metabolite production.
An in-vitro co-culture model utilising HepG2 liver spheroids as a source of metabolic enzymes has been developed, with several cytotoxic effects in MSC observed following chemotherapy treatment with alkylating prodrugs such as cyclophosphamide (CY). These include altered morphology, decreased expansion ($p<0.01$), and reduced expression of CD44 ($p<0.05$), an adhesion molecule involved in haemopoiesis. Similarly, treatment with active chemotherapeutics e.g. vincristine leads to grossly altered morphology, reduced CD44 expression ($p<0.01$) and decreased expansion ($p<0.001$). In the presence of liver spheroids, however, these effects are reduced ($p<0.05$ and $p<0.01$ respectively), indicating detoxification, as would occur in-vivo.
Genotoxic damage to MSC following CY treatment using the in-vitro model has also been studied using the COMET assay, with increases in DNA damage seen compared with MSC exposed to CY alone ($p<0.001$).
These results are comparable with effects seen in patients previously treated with chemotherapy for haematological malignancy. MSC expression of CD44 in these individuals is decreased ($p<0.05$), MSC survival in-vitro is reduced ($p<0.05$), ability of MSC to support haemopoiesis in-vitro is diminished ($p<0.05$) and an increase in genotoxic damage seen ($p<0.05$), even many years after completion of therapy. Therefore, in conclusion, a physiologically relevant in-vitro model has been developed to enable study of chemotherapeutic damage to MSC, which ultimately may enable treatment or prevention of this damage in the future.

P1012
Laser transmyocardial revascularization with autologous bone marrow cells: one-step method for harvesting and processing. Experience in 21 patients
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Background: Concentrated mononuclear cells obtained from autologous bone marrow harvest (ABMMC) may be an optimal source due to the mixed combination of stem, precursor and accessory cells to optimize the autocrine and paracrine effect of cell-based therapies. Recent studies suggest that LTMR with ABMC could improve the esults respect to laser alone.We evaluated a point of care device which utilizes density gradient centrifugation to concentrate bone marrow mononuclear cells for transmyocardial laser injection.
Patients and methods: 21 patients with diffuse coronary disease and medically refractory class III/IV angina (mean age 66 years old), were prospectively evaluated for cell-based therapy combined with transmyocardial revascularization (TMR). At the time of surgery, 120 cc of autologous bone marrow were aspirated from the posterior iliac crest and anticoagulated with citrate. Using a density gradient centrifugal system (HARVEST™, Palex, USA), bone marrow harvested was separated into its components (Figure), which included 20 cc of concentrated mononuclear cells inclusive of the buffy coat which was immediately available for direct transmural myocardial laser channels injection. Cell counts and flow cytometry were used to determine the total number of mononuclear cells in addition to specific somatic stem cell populations such as CD34+ and CD133+ cells.
Results: Time for bone marrow aspiration and concentration averaged 30 minutes. The complete processing was performed closed to the surgery room, in sterile ambient. There were no complications related to the bone marrow aspiration. Average cell counts pre and post concentration were significatively ($p<0.001$) increased (Table). No correlation between cell counts and patient demographics was detected. All patients received laser therapy with cell support without complications. 19 patients were evaluable for results.
Conclusions and comments: These preliminary data shows that density gradient centrifugation with the HARVEST™ device allows fast and efficient point of care concentration of ABMMC for cell-based therapies. Efficacy endpoints regarding cardiovascular applications and clinical results will be presented.

PATIENTS	Therapy	Post-Treatment	p
Functional angina	3.2 ± 0.5	1.2 ± 0.5	<0.001
Nº pills/month	330± 118	175± 92	0.001
Nitrates SL	22.1 ± 30.4	1.4 ± 1.3	0.02
Hospital admissions	3.8 ± 1.9	0.5 ± 0.8	<0.001

	MEDIAN CMN ($x10^6$/ml)	MEDIAN CD34+ ($x10^6$/ml)	MEDIAN CD133+ ($x10^6$/ml)
Cell therapy product (ABMC)	93.6 (43.7- 156.8)	0.8 (0.1 – 1.4)	0.36 (0.001 – 0.7)

P1013
Human dedifferentiated adipocytes show similar properties to bone marrow mesenchymal stem cells
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For many years, adipose tissue was regarded just as a heat insulator and a store of excess free fatty acids that could be released when needed. Now, it is considered as a critical organ involved in energy regulation, inflammation and immune response, through intricate signals. In previous work, we showed that the isolated adipocytes have some properties of pluripotent stem cells [1].

In the present study, we characterized the mature adipocyte isolated from human omental and subcutaneous fat tissues in the floating top layer by collagenase digestion and filtration. When mature adipocytes were subjected to in vitro ceiling culture, first lost their lipid droplets, then these cells were capable to change their morphology into fibroblast-like cells and they could successfully be maintained in culture. This morphological change was associated with functional properties. Indeed, dedifferentiated cells were able to inhibit the proliferation of stimulated lymphocytes in co-culture, while mature non-dedifferentiated adipocytes seemed to stimulate their growth. At the molecular level, as well as mesenchymal stem cells (MSCs) from bone marrow, dedifferentiated cells demonstrated to express HLA-A, HLA-DR and HLA-G, important features in immunoregolatory capacity.
Dedifferentiated cells, after trypsinization and reseeding, were able to proliferate in vitro, making a feeder layer and flow cytometric analysis revealed that these cells comprised a highly homogeneous cell population with the cell-surface antigen profile very similar to that of MSCs isolated from human bone marrow. Indeed, they stained positive for CD90, CD105, CD73, CD44, CD29 and negative for CD34, CD133, CD45 and CD117. Therefore, mature adipocytes have the potential to rapidly reverted to mesenchymal immunophenotype in vitro.
Embryonic stem cell genes that are required for self-renewal and pluripotency including Nanog, Oct4, Sox17, Gata4, Tbx1 were expressed at high levels, while there were no expression of Pax6 and Sox1.
In addition to bringing new insight into the biology of adipose tissue, these findings provide some informations on functionality and potential role of these cells.

P1014
Mesenchymal stem cells in cord blood: different from those in bone marrow?
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Introduction: Cord blood is one of sources of hematopoietic stem cell considered for clinical use in transplant procedures. Engraftment after cord blood transplantation is one of the major problems following this procedure. Mesenchymal stem cells (MSCs) are known as a marrow cells especially important for function of marrow stroma. However data regarding presence and function, e.g. cytokine secretion, of MSCs in cord blood are confusing.
Aim of the study: In the study we compared the numbers of CFU-F in cultures of MSCs from bone marrow and from cord blood and the concentration of cytokines: IL-10 and TNF-α in supernatant of mesenchymal stem cells cultures.
Material and methods: We included into the study ten frozen cord blood units obtained for research purpose from The Polish Stem Cell Bank. The results were compared with 19 samples of healthy bone marrow donors and their cultures. CFU-F cultures were performed according to research protocol and reagents provided by manufacturer (StemCell Technologies). Three cultures from each sample of bone marrow and cord blood were performed using 0,5 x 106, 1 x 106 and 2 x 106 mononuclear cells. Cytokines concentrations were measured in bone marrow plasma and in supernatant form bone marrow and cord blood CFU-F cultures using high sensitivity human immunoassay (R&D). Study was approved by Ethical Committee at Medical University in Lublin.
Results: Median number of CFU-F in bone marrow cultures were 2, 3 and 7 depending on number of cells in the culture. We could not observe in cord blood cultures any CFU-F colonies similar to those from bone marrow cells despite of using the same numbers of viable cells and the same medium and method of culture.
Median concentration of cytokines in supernatant and plasma are presented in the table.
We observed significantly higher concentration of IL 10 and TNF in supernatant MSCs cultures from bone marrow as compare to cord blood cultures ($p<0,001$). Significant increase of IL-10 and significant decrease of TNF concentration were noted in supernatant of culture as compare to plasma of bone marrow, which may be related to MSCs immune function.
Conclusion: Cord blood units seems to contain very little mesenchymal stem cells, capable to form CFU-F as compare to bone marrow. Absence MSCs cytokine activity in cord blood may contribute to different kinetics of stem cells engraftment and immune function.
Supported by grant of MNISW N407 117 35.

MSCs	Number of sources	Number of cultures	Median IL 10 [pg/ml]	Median TNF alpha [pg/ml]
Cord blood cultures	10	30	0,199877	0,163065
Bone marrow cultures	18	54	7,358055	0,363784
Bone marrow plasma	19	-	1,190657	1,45247

P1015
Cord blood banking – Perspectives for extended use
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In the last decade cord blood (CB) evolved from a “third line therapy” to the only stem cell (SC) source readily available for immediate use in patients needing an urgent hematopoetic SC transplant. From more than 500.000 units stored worldwide only a minor part is made available for pts reaching 1% of release p.a. Also, CB banks are not economically sustainable unless they release 2.5-3% of units p.a. becoming an economical burden to supporting institutions. WMDA analysis (2009) demonstrated that appr. 30% of pts need a double CB tlx meaning that 50% of transplanted units are supposed to be double cords. This requires a higher logistical effort for the matching strategy, the release and processing till tlx is performed and may cause the stagnant no. of cord blood tlx.
Cord blood is evolving to be a valuable cell source for non-hematopoetic indications and a variety of clinical studies is addressing the potential use in regenerative medicine, vascular diseases and metabolic disorders. In order to develop cell-based therapies for clinical use and future marketing under the challenging regulatory requirements (ATMP), close cooperation between academic institutions and biotech industry is mandatory to share costs and know-how and speed up the pharmaceutical development. One of the obstacles is the Informed Consent Form for CB donation which restricts the future use to established hematopoetic stem cell tlx and excludes potential indications. Once the unit is stored and released it is very difficult to come back to the donor (mom) to get an extended consent. Certainly the use for hematopoetic indications must be unrestricted. This may be accomplished by reserving large units with high TNC counts exclusively for the “classical” indications but allowing the majority of medium to low-sized units to be utilized in other indications after a predefined period of storage. CB banks may be reimbursed for storage and processing/release of CB units and supported in the maintenance of the bank. To define the conditions for expanded utilization of CB units in an ethical and rational manner, CB banks and registries need to adapt their structures and to improve the IT-based systems for effective search strategies. First estimations for the use in non-hematopoetic diseases indicate that public CB banks may become sustainable and capable of further banking and patients may profit from state-of-the-art cell-based tx.

P1016

NALP12 function as enhancer for MHC-I genes in patients undergo haematopoietic stem cell transplantation

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The reconstitution of the CD8 T lymphocyte in patients undergo hematopoietic cell transplantation (HCTs) depends largely of homeostatic peripheral expansion (HPE) of the mature lymphocytes that were not eliminated during the pre-transplant conditioning regimen or CD8 T lymphocytes that are contained within donors cells. It has been demonstrated that MHC-I molecules, IL-7 and IL-15 are required for an optimal HPE. In this context we have shown that the low and/or heterogeneous MHC-I levels on CD14 cells correlate with a slow and inefficient reconstitution of CD8 T lymphocytes in patients underwent allogeneic HPC transplantation. The deficiency in MHC-I expression could be due to a decrease in the expression of the NALP12 protein, which has been proposed as a positive regulator of classical and non-classical MHC-I genes. Therefore the aim of the project is to determine whether low levels of NALP12 correlate with downregulation MHC-I on monocytes from patients underwent allogeneic HPC transplantation. We studied a cohort of 11 patients, 6 of which received autologous transplant and 5 with alogeneic transplant. In 82% we observed reconstitution of CD14 within 30 days post-transplantation. Neither of the patients have reconstituted their T CD4+ population while most of the patients had normal levels of CD8 T lymphocytes. NALP12 levels were variable between each patient as well as during the first 150 days post-transplantation and show a positive correlation with the levels of MHC-I expression in both groups of patients.
In conclusion, our results suggest that NALP12 might function as an enhancer for MHC-I molecules expression, however we need to increase the numbers of patients to achieve conclusive results in that respect.

Cellular, gene therapies and Cytokines

P1017

Rapid adoptive T-cell therapy for resistant viral diseases

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Epstein-Barr virus (EBV), cytomegalovirus (CMV) and adenoviral reactivations are frequent complications after allogeneic SCT because of a lack of T cell control due to extensive immunosuppression. In some cases EBV may also transform into a malignancy called post transplantation lymphoproliferative disease (PTLD), with a high mortality rate. Cytotoxic T lymphocytes (CTLs) that recognize viral antigens are the most important immune effector mechanism controlling the persistent EBV, CMV and adeno infections.
First-line treatment for viral reactivation is dose reduction of the immunosuppressive drugs and/or anti-viral therapy. For PTLD this is followed by rituximab (anti-CD20 monoclonal antibody). Despite these multiple treatment strategies, the overall mortality from drug-resistant viremia after SCT is still considerable. Another treatment approach is adoptive transfer of virus specific CTLs from the donor. The standard method of adoptive T cell immunotherapy is laborious and time-consuming and is often too late to be administrated to the patient.
We have developed a clinical separation protocol for virus specific CTLs based on labeling with pentameric complexes containing recombinant HLA molecules together with virus derived peptides. By combining this labeling technique with a secondary magnetic sorting we have managed to get a purity of over 80% specific CTLs. This high purity diminish the risk of creating GVHD in the recipient even if the adoptive transfer of cells is done in an allogeneic or haplo-identical setting.
We first used this protocol in an 18 year old patient with life-threatening PTLD. The patient developed an EBV associated lymphoma involving lungs, liver and both kidneys and also showed extremely high EBV titers in blood. It was decided to give her EBV specific CTLs from her mother. 2 months after the given EBV specific CTL infusion the EBV associated lymphoma was in complete regression. After this we have further successfully treated five patients with life threatening viral disease (Adeno, CMV and EBV) with good efficacy. We could see a clinical and immunological response in four out of six patients. In one patient no monitoring could be performed from day seven until day 60. The patient is though alive and virus free up to date. In the 6th patient no response could be seen.
This method opens up the possibility to rapidly treat patients which are in acute need of T cell therapy and cannot wait for prolonged expansion techniques.

P1018

PD-1/PD-L1 interactions contribute to functional impairment of minor histocompatibility antigen-specific CD8+ T-cells targeting PD-L1-expressing leukaemic cells

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Leukemia relapses remain a serious problem after allogeneic stem cell transplantation (SCT), despite the long-term presence of minor histocompatibility antigen (MiHA)-specific memory CD8+ T cells, suggesting that these memory cells may lose function over time. Here, we investigated the role of Programmed Death-1 (PD-1)-mediated inhibition on MiHA-specific CD8+ T cells. We observed that myeloid leukemia cells express PD-L1, especially under inflammatory conditions. Furthermore, PD-L1 is highly upregulated on immature leukemic progenitor cells, whereas co-stimulatory molecules such as CD80 and CD86 are not expressed. This suggests that immature leukemic progenitor cells can evade the immune system by inhibiting T-cell function via the PD-1/PD-L1 pathway. Blocking PD-1-signaling using clinical grade human antibodies showed elevated proliferation and IFN-γ production of MiHA-specific cytotoxic T cells co-cultured with PD-L1-expressing leukemia cells. Furthermore, patients with relapsed leukemia after initial MiHA-specific T cell responses displayed high PD-L1 expression on CD34+ leukemia cells. In parallel, we observed increased PD-1 levels on MiHA-specific CD8+ T cells. Most importantly, we revealed that blocking PD-1/PD-L1 interactions augments proliferation of MiHA-specific CD8+ memory T cells. These data indicate that the PD-1/PD-L pathway can be hijacked as an immune evasion mechanism for myeloid leukemia. Blocking the PD-1 immune checkpoint is an attractive approach for post-SCT immunotherapy.

P1019

siRNA silencing of PD-L1 and PD-L2 on dendritic cells augments expansion and function of minor histocompatibility antigen-specific CD8+ T-cells

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Tumor relapse after HLA-matched allogeneic stem cell transplantation (SCT) remains a serious problem, despite long-term presence of minor histocompatibility antigen (MiHA)-specific memory T cells. Dendritic cell (DC)-based vaccination boosting MiHA-specific T cell immunity is an

appealing strategy to prevent or counteract tumor recurrence, but improvement is necessary to increase the clinical benefit. Here, we investigated whether knockdown of PD-L1 and PD-L2 on monocyte-derived DC results in improved T cell activation. Electroporation of single siRNA sequences into immature DC resulted in efficient, specific and long-lasting knockdown of PD-L1 and PD-L2 expression. PD-L knockdown DC strongly augmented IFN-γ and IL-2 production by stimulated T cells in an allogeneic MLR, while no effect was observed on T cell proliferation. Moreover, we demonstrated that PD-L gene-silencing, especially combined PD-L1 and PD-L2 knockdown, resulted in improved proliferation and cytokine production of KLH-specific CD4+ T cells. Most importantly, PD-L knockdown DC showed superior potential to expand MiHA-specific CD8+ effector and memory T cells from leukemia patients early after DLI and later during relapse. These data demonstrate that PD-L siRNA electroporated DC are highly effective in enhancing T cell proliferation and cytokine production, and therefore attractive cells for improving the efficacy of DC vaccines in cancer patients.

P1020

Editing human lymphocyte specificity for safe and effective adoptive immunotherapy of leukaemia

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T cell receptor (TCR) gene-transfer is an attractive strategy for the adoptive immunotherapy of tumors. However, the full potential of this approach is limited by a number of technical hurdles including inefficient gene transfer, unstable transgene expression, exhaustion of gene-modified cells and most importantly, co-expression of the endogenous and exogenous tumor-specific TCR in the same cell. The co-expression of endogenous and exogenous TCR causes not only reduced cell-surface expression of the introduced tumor-specific TCR, but also the potential for T cells to acquire autoreactive specificities due to mispairing between the different TCR chains. To address these limitations, we developed a novel strategy based on zinc finger nucleases (ZFNs) that allows for the first time the editing of T cell specificity at the DNA level, by combining the disruption of the endogenous TCR chain genes with the transfer of a tumor-specific TCR. Lymphocytes targeted by each set of ZFNs abrogated expression of the CD3/TCR complex on the cell surface. Sorted CD3neg cells proved stable in culture and permissive to lentiviral transduction. Indeed, introduction of exogenous TCR chains on a Lentivirus restored the expression and functionality of the CD3/TCR complex and allowed selective expansion of TCR-transduced cells by stimulation via the TCR complex. As a model TCR, we selected an HLA-A2 restricted, codon-optimized cysteine-modified TCR specific for the Wilms' tumor antigen 1 (WT1). For a complete editing of T cell specificity, we established a protocol that sequentially disrupted the endogenous TCR chains followed by lentiviral transfer of the WT1-specific TCR. This procedure resulted in a population of TCR-edited lymphocytes encoding only the tumor-specific TCR that, in the absence of competition, was expressed at high and physiological levels. Accordingly, TCR-edited lymphocytes were superior to conventional TCR-transferred cells in promoting specific recognition of WT1-expressing targets, including primary leukemias, and most importantly, were devoid of residual endogenous reactivity including alloreactivity. These data demonstrate that the successful genetic re-programming of T cell specificity in primary lymphocytes results in a functionally superior target specific killing activity and thus has the potential to greatly improve the safety and therapeutic benefit of cancer immunotherapy.

P1021

GMP-compliant simultaneous expansion and activation of NK and γ-delta T-cells with high cytolytic activity for immunotherapy

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Background and objectives: Adoptive transfer of NK cells seems to be a promising tool in cancer immunotherapy – alone or in combinations with other treatment modalities. Γ-delta T cells have also been shown to have antitumor activity in vitro and in vivo and – as NK cells – can be used in a haploidentical setting where the infusion of α-β T cells is strongly limited by the development of GvHD. We developed and evaluated a method to simultaneously expand NK and gd T cells under GMP conditions, tested cytotoxicity against cell lines and pediatric leukemic blasts and evaluated the tolerability of infusion of expanded haploidentical NK cells in 4 patients with relapse after prior haploidentical stem cell transplantation.

Methods: PMNC from healthy donors were expanded and activated for 14-21 days using a K562 cell line with cell surface expression of IL-15 and 4-1 BBL (as published by Imai et al, Blood 2005;106:376-83) and medium containing human AB serum and 100 IE/ml IL-2. After expansion enrichment of different cell subsets was done by MACS technology. Cell analysis was done by multicolor flow cytometry and cytotoxicity was tested with a BATDA release assay. For the clinical use of activated NK cells expanded cells were depleted from T cells by MACS technology.

Results: GMP compliant expansion (n=5) resulted in a 570 fold increase in NK cells (range 293-1034), and a 618 fold increase in gd T cells (range 168-1639). NK cells showed upregulation of different activating receptors (NKG2D, NKp30, NKp44, NKp46) after expansion whereas no difference was observed for the inhibitory KIR receptors. High cytotoxic activity was observed against K562 and leukemic cells. Patients received a median number of 56 x 10e6 cells/kg (range 15-80 x 10e6) without any signs of acute reactions or GvHD.

Conclusion: Our data indicate that expansion and activation of NK cells and γ delta T cells from haploidentical donors is feasible and that infusion of up to 80 x 10e6/kg of these NK cells is well tolerated in vivo. In an autologous or HLA-matched setting our modified method enable the infusion of both NK and γ delta T cells by use of the whole cell product. In combination with new technologies for depletion of α-β T cells, which are currently under development, infusion of NK and γ delta T cells will also be possible in the haploidentical setting.

P1022

IL-17-producing T-cells contribute to mediate acute graft-versus-host disease in patients undergoing unmanipulated blood and marrow transplantation

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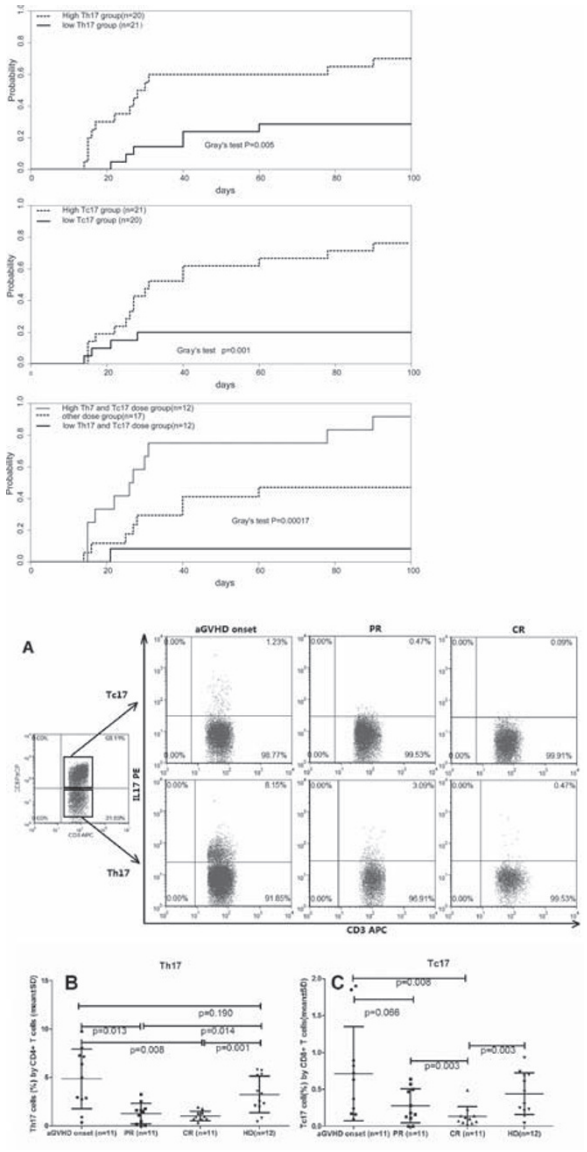
The aim of this study was to investigate the effects of IL-17-producing T cells, including Th17 and Tc17 cells, on acute graft-versus-host disease (aGVHD) in patients who had undergone granulocyte colony-stimulating factor (G-CSF)-mobilised peripheral blood stem cell (PBPC) and G-CSF-primed bone marrow (G-BM) transplantation. Allografts from forty-one patients were analysed for IL-17-producing T cells with respect to aGVHD. Furthermore, ten patients with aGVHD onset were monitored for the presence of Th17 cells in the peripheral blood by flow cytometry. Patients who received a higher dose of Th17 cells in the G-BM (>8.5×10^4/kg, p=0.005) or a higher dose of Tc17 cells in PBPC (>16.8×10^4/kg, p=0.001) exhibited a higher

incidence of aGVHD (figure1). An increased Th17 population (up to 4.99% CD4+ T lymphocytes) was observed in patients with aGVHD onset. In contrast, the percentage of Th17 population decreased drastically in aGVHD patients following treatment to achieve partial and complete remission (p=0.013 and p=0.008, respectively, figure2). All of the percentages of Th17 and Tc17 cells were significantly reduced after in vivo G-CSF application. Our results suggested that IL-17-producing T cells contributed to aGVHD. The application of G-CSF in vivo aided in reducing the occurrence of aGVHD through a decrease in IL-17 secretion by T cells.



P1023

Naturally occurring CD14+/HLA-G+ myeloid cells with in vitro immunosuppressive and immunotolerogenic properties in healthy individuals

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Introduction: HLA-G is a nonclassic MHC class I molecule that was initially found on trophoblasts, where it contributes to tolerance at the materno-fetal interface, while its expression in adults is restricted to thymus and cornea. HLA-G seems to regulate the rejection after solid organ transplantation but its role in allogeneic hematopoietic cell transplantation (HCT) is unknown.

Objectives: We have previously identified monocytic HLA-G positive cells (CD14+/HLA-G+) in peripheral blood (PB) of healthy individuals, which have shown to be HLA-DRlow. The aim of this study was to investigate their possible immunoregulatory and immunotolerogenic properties.

Methods: CD14+/HLA-G+ cells were isolated by FACS sorting and their immunoregulatory properties were analyzed by using them as third party cells in mixed lymphocyte cultures (MLC), with either unseparated PB mononuclear cells (PBMCs) or CD3+ cells as responders. The lymphoproliferative response in the cultures was measured using the CFSE cell proliferation assay. For test of the immunotolerogenic properties of the CD14+/HLA-G+ cells, CD3+ cells were preincubated with irradiated CD14+/HLA-G+ and then used in MLC, either as responder cells or irradiated third party cells. CD14+/HLAG+ pretreated CD3+ cells were analyzed for intracellular FOXP3 expression by FACS.

Results: FACS sorted CD14+/HLAG+ cells added as third party cells in MLC resulted in a dose dependent suppression of proliferation of responder PBMCs or responder CD3+ cells. The addition of the HLA-G neutralized Ab 87G reversed the suppressive effects. CD3+ cells that were pre-incubated with CD14+/HLAG+ cells for 18h or 4 days revealed decreased proliferation in MLC (17.2%) as compared to untreated CD3+ cells (56.7%), suggesting that CD14+/HLAG+ induces T-cell hyporesponsiveness. Besides that, CD14+/HLAG+ pretreated/ CD3+ cells found to suppress the lympho-proliferation in MLC when added as third party cells, suggesting that the pretreated CD3+ cells became immunosuppresive. These CD14+/HLAG+ pretreated/ CD3+ regulatory cells were FOXP3 negative. Interestingly, incubation of CD3+ cells with the CD14+/HLAG+ cells resulted in loss of CD4 expression.

Conclusions: Naturally occurring HLA-G+ monocytic cells exhibit in vitro immunosuppressive function and induce hyporesponsivess and regulatory properties on T cells. These cells might prove a promising strategy for adoptive cell immunosuppressive therapy after allo-HCT.

P1024

Early apoptotic cells cellular treatment in allogeneic haematopoietic cell transplantation

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Over the past decade, advances have been made in the care of patients undergoing hematopoietic stem cell transplantation (HSCT). However, probability of death by day 200 not proceeded by relapse among patients who received myeloablative regimens, remains high in the last decade.

We have developed a cellular therapy, ApoCell, prepared by a donor leukopheresis followed by mononuclear freezing, and thawing 30 hours before HSCT. The thawed cell are exposed to specific incubation conditions that allow the preparation of large amounts of ApoCell, mononuclear donor apoptotic cells that in average contains 60% of early apoptotic mononuclear cell with less than 3% propidium iodide positive cells (necrotic cells). This preparation is stable for 24 hours without going into secondary necrosis. ApoCell showed in vitro, tolerizing effect to dendritic cells and macrophages exposed to LPS, with significant ($p<0.001$) inhibition of pro-inflammatory cytokines; IL-1b, IL-12, and IL-6, and significant ($p<0.001$) downregulation of MHC-Class II, CD40, and CD86. ApoCell was tested in vivo, in mice model of GvHD and showed significant rescue effect and amelioration of overall survival ($p<0.05$). In another model that included leukemia induction, no failure in graft versus leukemia was seen in mice that received ApoCell, and leukemia was prevented as in GvHD control. In a third type of experiment, ApoCell was given along with cyclosporine and methotrexate,

representing the most common standard of care, and showed synergistic anti-GvHD effect.
Taken together, ApoCell is a promising stable apoptotic cell preparation for the prevention of GvHD and for decreasing non-relapse associated death. Enlivex LTD, have initiated a phase 1/2a, multicenter, open-label study designed to evaluate the safety, tolerability and preliminary efficacy of ApoCell administration for the prevention of acute GvHD in subjects with hematologic malignancies undergoing allogeneic sibling HLA-matched hematopoietic HSCT. Six patients were so far recruited.

P1025
Ex vivo induction of multiple myeloma-specific T-cell responses by dendritic cells stimulated by whole-tumour antigen of autologous myeloma cells
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Dendritic cells (DCs) of patients with multiple myeloma (MM) are functionally defective and fail to induce tumor-specific immune responses in vivo. Stimulation of DCs by MM-associated antigens ex vivo may overcome suppression induced by tumor microenvironment. Loading of DCs with the total antigenic array of myeloma cells is preferable to the idiotype which is weakly immunogenic. The study objective was the stimulation of DCs from MM patients by autologous tumor antigens, and the assessment of specificity of the in vitro induced T cell responses. Twenty patients with MM at diagnosis or relapse were enrolled in the study. DCs were produced by culture of peripheral blood monocytes in the presence of GM-CSF and IL-4, and were tested by flow cytometry and allogeneic mixed lymphocyte reaction (MLR). Autologous myeloma cells (AMC) were isolated from bone marrow aspirates using anti-CD138 magnetic microbeads. DCs were stimulated either by phagocytosis of apoptotic bodies from irradiated AMC or transfection with total RNA of AMC by electroporation (in 14 and 7 cases, respectively). After TNFa-induced maturation, stimulated DCs were cocultured with autologous lymphocytes in the presence of IL-2. The specificity of the in vitro expanded T cells was assessed by colorimetric cytotoxicity assay against AMC targets or by ELISpot technique for the enumeration of cells that secrete IFN-g after incubation with AMC (in 16 and 5 cases, respectively). In vitro generation of DCs with normal immunophenotype and function was feasible in all patients. With optimization of electroporation conditions, the rate of viable DC transfection reached 80%. A significant CD8+ T cell-mediated cytotoxic activity against AMC (>10%) was detected within the in vitro expanded lymphocytes (Figure 1). Induction of anti-MM specific cytotoxic T cells was successful in 9 out of 11 cases by loading of DCs with apoptotic bodies, and in 4 out of 5 cases by transfection of DCs with RNA. ELISpot analysis revealed the presence of CD4+ and CD8+ T cell-mediated specific responses, that were induced either by apoptotic body-stimulated (3 out of 3 patients) or by RNA-loaded DCs (2 out of 2 patients). The frequency of IFN-g-producing T cells was 70-141 and 20-60 per 10^5 cells, respectively. In conclusion, ex vivo stimulation of DCs from MM patients by whole tumor antigen load is effective in the induction of MM-specific immune responses, and may provide a platform for the design of immunotherapy protocols.

P1026
Memory T-cells of CMV-negative donors are superior recipients of CMV-specific T-cell receptor RNA
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Cytomegalovirus (CMV)-associated disease is a life-threatening complication after allogeneic hematopoietic stem cell transplantation (HSCT). Long-term virus control requires the re-establishment of protective antiviral T cell immunity in the host. The latter is challenging, particularly if the donor is CMV-negative and thus, no CMV-reactive T cells are being transferred to recipient during HSCT. Grafting nonreactive T cells of CMV-negative donors by virus-antigen specific T cell receptors (TCR) may be an efficient means to transfer CMV specific T cell function into HSCT recipients. In this study, we have reprogrammed T cells of CMV-negative donors by transfer of human in vitro transcribed TCR RNA recognizing the immunodominant HLA-A*0201-binding CMVpp65 epitope 495-503 (TCRpp65). This procedure resulted in transient expression of the introduced TCR for up to one week. Because of the instability of introduced RNA molecules a conceivable study protocol would aim at weekly administration of TCR redirected T cells. However, this approach may be hampered by the induction of serious alloreactivity through the repeated transfer of polyclonal donor T cells with unknown endogenous specificity.
To address this concern, we generated TCRpp65 transfected pure naïve and memory T cell subsets. The latter have been reported to induce less alloreactivity due to a more restricted endogenous TCR repertoire. Although both naïve and memory T cell subsets showed comparable expression of TCRpp65 RNA, memory CD8+ T cells mediated superior cytotoxicity against CMV-infected fibroblasts for up to one week. Alternatively, we generated EBV/HLA-A*0201 peptide-specific T cell lines and transfected them with TCRpp65 RNA to obtain EBV/CMV-bispecific T cells. As with TCR redirected memory T cell subsets, EBV/CMV-bispecific CD8+ T cells showed strong reactivity against CMV-infected fibroblasts for up to one week without hampering the endogenous EBV peptide-specific effector function. Studies to compare the alloreactive potential of both memory T cell subsets and EBV-specific T cell lines are ongoing. In summary, our data demonstrate that memory T cells from CMV-negative donors can be easily redirected with TCRpp65 RNA, thereby gaining CMV-specific T cell

[P1025]

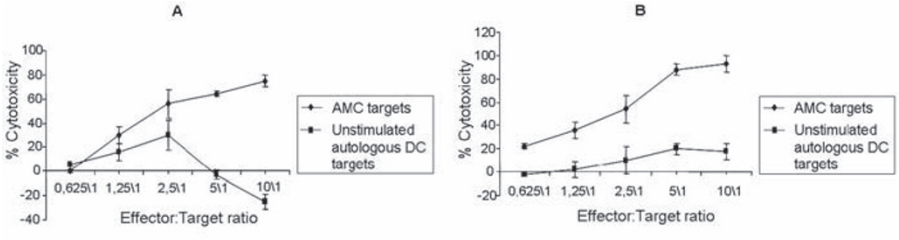

Figure 1: MM-specific cytotoxic activity of *in vitro* expanded lymphocytes. (A): CD8+ effector T cells induced by apoptotic body-loaded DCs. (B): CD8+ effector T cells induced by RNA-transfected DCs. Each cytotoxicity percentage represents the mean value ± standard deviation of triplicate wells.

effector function for a considerable time period. We believe that TCRpp65 RNA has the potential to be further developed as a therapeutic 'off-the-shelf' reagent for CMV-positive patients who undergo allogeneic HSCT from CMV-negative donors.

P1027

Massive expansion of umbilical cord derived mesenchymal stromal cells (MSC): an innovative and unlimited source of MSC with potent immunosuppressive activity for the treatment of GvHD

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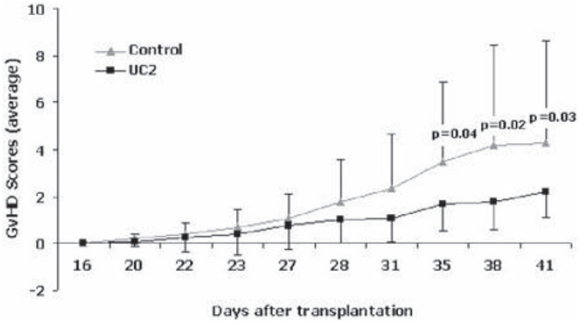
Background: Mesenchymal Stromal Cells (MSC) have been recently identified as a therapeutic option in several clinical conditions. Whereas bone marrow is considered the main source of MSC (BM-MSC), the invasive technique required for collection and the decline in the allogeneic donations call for alternative sources. Human umbilical cord (UC) represents a painless, non-controversial and easily accessible source of MSC (UC-MSC).

Methods: Sections of full-term UC were mechanically disaggregated and fragments obtained were directly transferred to cell culture flasks and cultured in 5% human platelet lysate (PL)-enriched medium. Two isolation techniques were compared which differ for the presence ("wet" method) or absence ("dry" method) of the culture medium during the "fragmentation phase" of the umbilical tissue. Neither enzymatic digestion nor blood vessels removal were performed. After two weeks, the adherent cells were harvested (P1), re-plated at low density and expanded for two consecutive rounds (P2 and P3).

Results: We successfully isolated and expanded MSC from 9/9 UCs. In 7+1 days, UC-MSC expanded with a mean Fold Increase (FI) of 212+50 from P1 to P2 and with a mean FI of 206+65 from P2 to P3 in additional 9+1days. Applying our current standard expansion protocol for BM-derived MSC expansion we had theoretically reached approximately $1.0x10^9$ cells at the end of P3. Moreover, if all P1 cells from processing "wet" method had been seeded, we have estimated that a median of $9.5x10^{10}$ (range from $1.0x10^{10}$ to $29.0 x10^{10}$) would have been theoretically obtained from each single cord "wet" processed. UC-MSC were characterized in comparison to BM-MSC: they expressed the same standard surface markers and they showed the same immunosuppressive activity in an in vitro lymphocyte proliferation assay but they contained more CFU-F and seemed to be less committed towards osteogenic and adipogenic lineages than BM-MSC. Both array-CGH analysis and karyotyping revealed no chromosome alterations at the end of the expansions. Animal studies revealed no tumorigenicity in vivo. Interestingly we could also demonstrate here for the first time an immunosuppressive capacity of UC-MSC since these cells very efficiently delayed the insurgence of cGvHD in an in vivo mouse model for this disease (Fig.1).

Discussion: UC constitutes a convenient and very rich source of MSC for the production of third-party "clinical doses" of cells under GMP conditions.



P1028

Expansion of cord blood-derived T-cells for use as predictive tool of cord blood transplantation complications and outcome

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Umbilical cord blood (CB) is an alternative source of hematopoietic stem cells for allogeneic stem cell transplantation when no HLA-identical adult donor is available. However, hesitation to use CB remains since this stem cell source offers no possibility for donor lymphocyte infusions (DLI) after transplantation. We have previously reported the successful expansion of functional T cells from given grafts (Okas et al J Immunother. 2010 Jan;33(1):96-105). In the present study we have analyzed if the T cell expansion might work as a prognostic tool for in vivo complications after transplantation. We have used multi color flow cytometry to correlate in vitro phenotypical data and expansion potential from 32 expansions to the clinical outcome of the transplantations. Our results indicate that a poor expansion of T cells in vitro is associated to clinical graft rejection (p=0.026). Furthermore, higher frequencies of CD4+CD25+FoxP3+ regulatory T cells (p=0.049) and lower frequencies of both CD4+CD25+ (p=0.015) and CD8+CD25+ (p=0.028) activated T cells in the expansion were associated with an increased risk of relapse. A low percentage of a certain subset of CD8+ T cells which dimly co-express CD4 on their surface was associated to sepsis (p=0.033), whereas a high percentage of γ delta T cells was associated to clinical relapse (p=0.038). Finally, high frequencies of total CD45RO+ memory cells were associated to both skin and liver graft versus host disease (p=0.003). In conclusion, our data would suggest that expansion of CB T cells from the graft may not only be used as future DLI to treat malignant relapse or threatening rejection, but also as an in vitro indicator which might give essential information of how to manage CB transplanted patients.

P1029

Plasmacytoid dendritic cells play a crucial role in apoptotic leukocyte-induced immune immunomodulation

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Apoptotic cells have demonstrated their immunomodulatory properties in several clinical and experimental situations associated with allo- or auto-reactive conflict. Apoptotic cells by themselves can prevent and/or down-regulate immune responses through immunomodulatory cytokine release. Antigen presenting cells (APC) and professional phagocytes play an important role to amplify and maintain apoptotic cell-related immunodulatory properties mainly through anti-inflammatory factor secretion including IL-10 and TGF-β. Several APC have been implicated in immunomodulatory effects associated to apoptotic cells. However, the role of plasmacytoid dendritic cells (pDC) in apoptotic cell-induced immunomodulation has

not yet been evaluated. We used an experimental model of allogenic bone marrow (BM) transplantation in which apoptotic cell injection both controls the alloreactive conflict allowing engraftment and favors regulatory T cell (Treg) increase. In such model, we observed that pDC (but not conventional DC) within the BM donor graft played a crucial role in apoptotic cell-induced engraftment. Their depletion using anti-mPDCA-1 specific antibody treatment inhibited both apoptotic cell-induced BM engraftment and Treg increase. To determine the mechanism, we first cocultured enriched pDC with apoptotic cells. Surprisingly, pDC were not directly affected by apoptotic cells. Coculture of pDC with apoptotic cells did not modify CpG ODN2216-induced pDC maturation (higher CD86 and IAIE expression) and IFN-α production. However, supernatant issued from macrophages cocultured with apoptotic cells significantly prevented CpG-induced pDC maturation and IFN-α production. To confirm phagocyte involvement, we injected naïve mice with apoptotic cells. Ex vivo, pDC issued from mice having received apoptotic cells were refractory to CpG stimuli in contrast to pDC issued from mice receiving PBS. This effect was inhibited when macrophages were depleted prior apoptotic cell injection using clodronate-loaded liposome injection. More interestingly, pDC issued from mice having received apoptotic cells induced Treg commitment compared to pDC issued from control mice. As expected, clodronate-loaded liposome injection indeed significantly inhibited pDC-induced Treg polarization. Overall, this suggests that apoptotic cells indirectly induce BM pDC with tolerogenic properties that are necessary for Treg generation and immunomodulation of the allo-reactive conflict.

P1030

Intramyocardial cellular therapy with CD133 cells asociated with coronary bypass

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Introduction: Cellular therapy (CT) has been evaluated in the cardiology field with contradictory results. AIMS: To evaluate the benefits of intramyocardial CT using CD133+ cells in patients undergoing surgical revascularization; and to determine the associated risk and secondary benefits.
Material and methods: Prospective nonrandomized study, approved by the ethical committee of the centre. Inclusion criteria: patients older than 18 y/o, history of myocardial infarction with more than one month of history, one or more akinetic/dyskinetic zones by SPECT or MRI, with left ventricular ejection fraction (LVEF) between 30-40%. CD133+ cells were injected intramyocardically at the time of the surgical revascularization. For mobilization, filgrastim was administered for 4 days. On day 5, stem cells mobilized were collected using the COBE Spectra Apheresis System, processed by the COBE 2991 Blood Cell Processor and then incubated with the anti-CD133 antibody. The final cellular preparation was processed by the CliniMacs device for the purification of the CD133+ cells. The count of CD133+ cells was performed by flow cytometry with the monoclonal antibody anti-CD133/2 [293C3]. The CD133-enriched fraction was diluted in autologous plasma, preserved in continued mixed state for 24 hours, concentrated by centrifugation before intramyocardial injection and re-suspended in syringes containing 2ml, in sterile conditions.
Results: 14 cases and 14 controls were included. Mean follow-up time was 21 months. Both groups were balanced respect age, comorbidities, coronary disease and basal LVEF. Patients who received CT showed less post intervention inflammatory response evaluated by PCR and had less renal function impairment in the near post intervention and at the discharge. CT was not related with increased myocardial damage evaluated by troponin determination. CT was associated with an increase of 8% of the LVEF whereas in the control group, the LVEF decreased 1.3%. At six months, there was an improvement of the contractility in the segments treated with CT compared with the control group (p: 0.006). There weren't complications related with the procedure with a follow-up time of 14 months.
Conclusion: Intramyocardial cellular therapy is a secure procedure that seems to confer benefits to the cardiac function and probably in other orders as for example renal function. Longer follow-up time and a larger number of patients are needed to confirm these data.

P1031

Successful generation of human herpesvirus 6 (HHV6)-specific T-cell lines for treatment of HHV6-related complications after 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, no effective specific antiviral treatment for HHV6 reactivation has been identified, and patients with HHV6-related disease were mostly treated with ganciclovir, with suboptimal results. T cell therapy has been demonstrated as a unique opportunity to restore antiviral immune surveillance after HSCT, leading to clearance of infection and prevention/treatment of disease.
We conducted scale-up experiments to validate a method of in vitro culture to expand T cells specific for HHV6 from 14 HLA-haploidentical HSCT donors, by peripheral blood mononuclear cell (PBMC) stimulation with a pool of 15-mer peptides derived from the HHV6 type 6B U54 protein. T-cell lines, that included a majority of CD4+ T lymphocytes, were successfully generated from 13 of 14 individuals. Specific INFγ secretion was measured in Elispot assays. The T-cell lines showed specific INFγ production consistently higher (median 89 SFU/105 cells, range 0-508) than non-cultured donor PBMC (median 2 SFU/105 cells, range 0-49). In a standard 51chromium release assay, 12 of the 13 T-cell lines presented specific cytotoxic activity against HHV6 (median 13%, range 4-83), with only 1/12 showing residual alloreactivity. The lysis observed was mainly HLA class II-restricted, thus CD4+ T-cell mediated. One of the two T-cell lines showing lower specific cytotoxic activity and the T cell line that did not display specific cytotoxicity showed high INFγ production when tested in an ELISPOT assay (484 and 170 SFU/105 cells, respectively). Our data indicate that HHV6-specific CD4 T-cell lines with an efficient in vitro antiviral response and low/undetectable alloreactivity against recipient targets may be expanded from PBMC of most HSCT donors after stimulation with HHV6 U54 protein-derived peptides. The efficacy of these T cells for pre-emptive or curative treatment of HHV6-related complications remains to be evaluated in clinical trials.

P1032

Detailed monitoring of selective graft versus leukemia T-cell immune reactions following donor lymphocyte infusion

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Allogeneic stem cell transplantation is a curative treatment for patients suffering from hematological malignancies. In case of recurrent disease, donor lymphocytes infusion (DLI) can elicit an immune response specifically targeting residual patient cells resulting in graft versus leukemia (GVL) reactivity, but also in graft versus host disease (GvHD). Differential effects of DLI can range from no response to long lasting complete remissions in the absence or presence of GvHD.

Previously, we demonstrated high frequencies (30%) of circulating activated T cells targeting various minor histocompatibility antigens (MiHAs) responsible for both GVL and GvHD in patients responding to DLI in the presence of limited, but recognizable GvHD. To selectively identify beneficial immune responses, we analyzed CD8+ T cell responses in 5 CML patients that achieved continuous molecular remissions after DLI without any sign of GvHD. From samples taken prior to DLI, and at various time points after DLI during conversion to full donor chimerism and disappearance of the BCR/ABL signal, CD8+ T cells were single cell sorted using HLA-DR as an activation marker. After expansion, 72±52 CD8+ T cell clones were obtained per sample. Interestingly, recognition of both patient and donor derived EBV-LCL was observed in 24±14% of the clones, illustrating ongoing T cell immunity against EBV. On average 5±5% MiHA specific reactive T cell clones, as determined by selective recognition of patient derived target cells, but not donor cells, were found at specific time points after DLI. Whereas the level of MiHA reactivity in these samples was low, the presence of MiHA specific T cells coincided with increasing donor chimerism and decreasing BCR/ABL signals. Moreover, no MiHA reactivities were detected in pre DLI samples and in cloned CD8+ T cells that did not express HLA-DR. Our data demonstrate that only a minority of activated T cells in patients responding to DLI with GVL reactivity in the absence of GvHD plays a role in the specific recognition of patient derived hematopoietic cells. Although peak GvL responses may have been missed due to the absence of GvHD in some patients, we conclude that the relative small contribution of MiHA specific T cells is likely to be responsible for the gradual beneficial immune response in these patients. Molecular characterization of these antigens using whole genome association scanning will reveal the relevant specificities of these T cell responses.

P1033

Lentiviral vector enhanced haematopoietic stem cell gene therapy for mucopolysaccharidosis type IIIA

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MPS IIIA is a lysosomal disorder caused by deficiency of sulfamidase (SGSH), catabolising heparan sulphate (HS). Neither enzyme replacement therapy, nor haematopoietic stem cell transplantation (HSCT) correct the progressive neuropathy. We propose this is due to a dose effect, where insufficient enzyme is produced by donor-derived brain microglial cells after HSCT. We compare HSCT using normal cells (WT-HSCT) against lentiviral overexpression of SGSH in transplanted haematopoietic stem cells (LV-HSCT) in MPS IIIA mice.
Lineage depleted bone marrow was transduced with an SGSH-lentiviral vector. Transduced or untransduced cells delivered to myeloablated MPS IIIA mice standardly achieved >90% chimerism. Mice were perfused with Tyrodes and sacrificed 6 months post-transplant.
Six months after WT-HSCT we find 61% of normal (WT) SGSH activity in the spleen, 39% in liver and no significant increase in brains of MPS IIIA recipients. In contrast, recipients of LV-HSCT showed 100% of WT in spleen, 48% in liver and a significant increase to 10% in brain. In the cerebral cortex after WT-HSCT, lysosomal size is not significantly reduced, whilst neuroinflammation (microglial cell numbers) is significantly reduced to 54% of MPS IIIA levels. LV-HSCT significantly reduces cortex lysosomal size to 12%, with normalisation in some animals and neuroinflammation to 41% of MPS IIIA levels. MPS IIIA mice display increased hyperactivity and rapid exploratory behaviour and reduced anxiety and immobility in the open field test. WT-HSCT was unable to significantly change any of these parameters whilst LV-HSCT normalised all of them to WT levels.
The enzyme levels achieved, the reduced lysosomal size, neuroinflammation, and behavioural correction suggest that LV-HSCT may be a clinically viable approach to treat MPS IIIA where WT-HSCT is ineffectual.

P1034

Simultaneously isolation of multi-specific and multi-functional T-cells product for treatment of patients undergoing allogeneic haematopoetic stem cell transplantation

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Allogeneic hematopoetic stem cell transplantation (aHSCT) is the treatment option for a variety of diseases in particular heamatological malignancies. Due to an ongoing immunosupression to prevent graft versus host reaction (GvHD), viral infections as well as disease relapse are the major causes of morbidity and mortality after aHSCT.
Cytomegalovirus (CMV) and Epstein-Barr-Virus (EBV) persist in a latent phase following infection. Reactivation causes significant morbidity and mortality after aHSCT, in particular CMV pneumonitis and EBV associated post transplant lymphoproliferative disease (PTLD).
Adenovirus (AdV) is a common opportunistic pathogen causing serious infectious complications especially among children after aHSCT.
Moreover, not all of these opportunistic infections are susceptible to antiviral therapeutic.
One way to overcome the limitation of antiviral therapy is through adoptive transfer of viral specific cytotoxic T lymphocytes (CTLs).
With a newly established protocol, based on streptamer selection, we isolated simultaneously multi functional and multi specific T-cells, namely against CMV, EBV and AdV, from a single blood donation. In this simultaneous selection, even the AdV specific T cells, which are known to be rarely detected among healthy donors, could be enriched to an amount sufficient for a direct T cell transfer.
Purity achieved after selection was at least 85% and up to more than 95%, which minimizes the risk for GvHD after clinical application.
Furthermore, the selected multi specific T cells could be expanded in vitro without loss of specificity and include different phenotypes such as central memory and memory effector cells, which may provide long lasting immunity.
Moreover, starting with a leukapheresis, we successfully transferred our selection protocol in a closed system conforming to current good manufacturing practice (cGMP) requirements, which allows clinical application in the future. With that, it opens new perspectives in cellular immunotherapy either against viral infection or malignancies for patients after aHSCT.

P1035

Good manufacturing practice grade mesenchymal stromal cells isolation and expansion in an animal component free functionally closed bioreactor system

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Human MSC can be derived from BM in limited numbers, making ex-vivo expansion necessary. Safety standards for expansion systems of clinically applicable cells render animal component free, GMP-grade expansion obsolete. We established lysates of clinical grade platelet concentrates (PL) as an appropriate good manufacturing practice (GMP) compliant

supplement. PL was used with the semi-automated, functionally closed bioreactor system QUANTUM Cell Expansion System from CaridianBCT Inc. for large-scale two-step isolation and expansion of MSC. The surface of the bioreactor is 21.000 cm^2 and has to be coated to enable adherence of cells. We used 100–300 mL of PL for coating. In a first step, BM was collected by iliac crest puncture (n=4). Aspiration volume was 22±5 mL with a total number of colony forming units of fibroblasts (CFU-F) of 82±66x10E3. The whole BM was loaded onto a bioreactor at a seeding density of 7±2x10E3 mononuclear cells/cm^2. After 14 days 39±27x10E6 MSC were harvested at a density of 2±1x10E3 per cm^2, i.e. 10±1 population doublings. Doubling time was 36±7 h. In a second step, 17±4x10E6 MSC (i.e. 801±234 MSC/cm^2) of the harvest were loaded on another pre-coated bioreactor. After 6 days of culture, a total of 439±342x10E6 MSC/BM (97±4% viability) at a density of 7±2x10E3 MSC/cm^2 was harvested from this second expansion step. The doubling time was 46±9 h, number of population doublings was 3±1. MSC harvested from passage 1 showed adipogenic, chondrogenic and osteogenic differentiation potential and were positive for CD73, CD90, CD105 and HLA A,B,C but negative for CD3, CD34, CD45 and HLA DQ,DP,DR. MSC pre-expanded in the bioreactor system were used to test different surface coatings of the bioreactor. Fibronectin was the most suitable coating reagent, followed by PL and human frozen plasma, whereas poly-L-lysine did not support growth. Both freshly expanded (n=8) and cryopreserved cells (n=25) were expanded successfully on the bioreactor. Our data show, that the QUANTUM system is a functionally closed tool for GMP compliant expansion of MSC. Cells expanded in a two-step protocol using this bioreactor fulfil criteria of MSC regarding their differentiation capacity and standard surface marker expression profile. Moreover, the bioreactor system is easy to use and allows (in combination with GMP-grade PL) isolation and expansion of GMP grade BM-derived MSC outside a GMP facility without use of components of animal origin.

P1036

Alloreactive and leukaemia-reactive T-cells mainly derive from naïve precursors in healthy donors: implications for immunotherapy with memory T-cells

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HLA-mismatch antigens are major targets of alloreactive T cells in HLA-incompatible stem-cell transplantation. They can trigger severe graft-versus-host disease (GvHD) and reduce survival in transplant recipients. Previous work in murine models has demonstrated that GvHD-mediating T cells mainly derive from the naive T-cell compartment. Therefore, depletion of naive T cells may be a relatively easy strategy to prevent GvHD also in humans.
Here we screened CD4 and CD8 T-cell subsets sorted from peripheral blood by flow cytometry according to expression of naive and memory markers CD45RA, CD45RO, CD62L, and CCR7 for alloreactivity to individual HLA-A/-B/-C/-DR/-DQ mismatch alleles in mixed lymphocyte reactions (MLR) in vitro. Subsets were defined by a single naive (or memory) marker to facilitate translation into a clinical-grade allodepletion procedure. As alloantigen-presenting cells, we used HLA-deficient K562 cells transfected with cDNA or RNA of single disparate HLA class I or II alleles.
We observed in IFN-g ELISPOT assays of day 12-old MLR responders that allo-HLA reactivity preferentially derived from subsets enriched for naive compared to memory T cells in healthy donors (n=15), irrespective of the HLA mismatch allele. This separation was most efficient if CD45RA (versus other markers) was used to sort naive and memory subsets. Median numbers of allo-HLA-reactive effector cells were 4.4-fold and 11.5-fold lower in CD45RAneg memory CD8 and CD4 T cells than in entire CD8 and CD4 T cells, respectively. In allele-specific analysis, alloreactivity to single HLA-A/-B/-C/-DR alleles clearly exceeded that to HLA-DQ alleles. This result reflected the clinical observation that HLA-DQ mismatches have a lower GvHD potential compared to other disparate HLA antigens. We also demonstrated in HLA-matched donor-patient pairs that leukemia-reactive CD8 cytotoxic T-lymphocytes mainly derived from subsets enriched for naive compared to memory T cells.
We concluded that 'naive-depleted' T-cell subsets of healthy individuals showed decreased allo-HLA reactivity, but lacked significant anti-leukemia responses in vitro. The clinical use of 'naive-depleted' (or memory) T cells (e.g. CD45RAneg) might be beneficial for HLA-mismatched patients at high-risk of GvHD and low-risk of leukemia relapse. Preferred allografts are those which contain leukemia-reactive memory T cells. Alternatively, replenishment with leukemia-reactive T cells isolated from naive subsets is desirable.

P1037

Stimulating surface molecules, Th1 polarizing cytokines, proven trafficking – A new protocol for the generation of clinical grade dendritic cells

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Dendritic cells (DC) are the most potent antigen presenting cells (APCs) regulating T cell-based immunity against infections and malignancies. Effective induction of cell-mediated immunity strongly depends on the ability of DC to: (a) migrate to draining lymphoid organs mediated by chemokine receptors, (b) prime T cells through high expression of costimulatory molecules and MHC- complexes, (c) secret Th1-polarizing cytokines such as Interleukin-12 (IL-12). Only fully matured DC are able to induce a specific T cell response, whereas immature DC tend to induce tolerance and expand regulatory T cells that may jeopardize the effectiveness against viral- or tumor-specific T-cell immunity.
DC have been vigorously investigated as immunological basis for therapeutic vaccination against cancer and infections, even amomg patients after allogeneic stem cell transplantation. But still, there is no protocol to generate fully matured and functional DC according to methodical requirements of current Good Manufacturing Practice (cGMP) guidelines.
For the first time, we established a protocol conforming to cGMP requirements which permit the generation of fully matured and functional DC. Our protocol is based on cell culture in adherence bags using serum free media, and a maturation cocktail, containing tumor necrosis factor-α (TNF-a) / Interferon-α (IFN-a) / polyinosinic:polycytidylic acid (poly-I:C).
DC generated according to our new protocol superiorly displayed three critical features for an effective induction of cell-mediated immunity without evidence of exhaustion as compared to conventional produced DC with: (1) high surface expression of MHC- and costimulatory molecules as well as homing receptors, (2) secretion of Th1-polarizing cytokines, (3) active migratory potency. Moreover, our DC induced high frequencies of functional CMV specific T cells after stimulation with pp65 peptide pool in vitro.
We demonstrate that intracellular changes linked to cell adherence are important and significantly impact DC function. Adding poly-(I:C) with TNF-a for maturation skews the DC towards Th1-polarisation and IFN-a synergizes with poly-(I:C) and TNF-a to allow generation of DCs in serum free condition.
Our newly developed protocol offers an easy method to produce fully matured Th1-polarizing DC with proven migratory and stimulatory capacity for any clinical application.

P1038

Feasibility of very low dose donor lymphocyte infusion early after T-cell depleted allogeneic stem cell transplantation

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T cell depletion (TCD) effectively reduces the mortality due to graft-versus-host-disease (GvHD) after allogeneic stem cell transplantation (SCT), albeit at the cost of an increased relapse risk. Graft-versus-tumor responses can be achieved with limited GvHD later after TCD-SCT with donor lymphocyte infusions (DLI). Since the interval between DLI and SCT is inversely correlated with the severity of DLI-induced GvHD, we administer DLI at a low dose of 3x10E6 CD3+ T cells/kg at 6 months after SCT. However, DLI at an earlier time point would be preferable to prevent early disease relapse after SCT in patients with high risk malignancies.

To study the toxicity and efficacy of very low dose DLI early after SCT, we prospectively administered 0.3x10E6 CD3+ T cells/kg from related or 0.15x10E6 CD3+ T cells/kg from unrelated donors to 20 patients scheduled at 3 months after SCT. All patients had high risk disease (12 AML, 2 ALL, 1 plasma cell leukemia, 2 T-cell NHL, 1 blastic NK cell lymphoma, 1 MCL, 1 CMML). Ten patients had been transplanted with a reduced-intensity, 10 patients with a myeloablative regimen. Alemtuzumab was added to all grafts for in vitro TCD. Seven patients were transplanted with a related and 13 with an unrelated donor. At the time of DLI, 16 patients were in complete remission and 4 had progressive disease. DLI was given at an actual median of 120 (range 90-140) days after SCT.

Two patients died within 3 months after DLI without GvHD. In the remaining 18 patients, four patients (1 transplanted with a related, 3 with an unrelated donor) developed acute GvHD grade 1-2 of the skin and one additional patient grade 4 GvHD of the liver at a median of 28 days after DLI (range 18-91). Acute GvHD necessitated systemic therapy in three patients and resolved in all patients. Among ten patients with mixed bone marrow chimerism before DLI, 5 patients converted to full donor chimerism as defined by ≤1% donor DNA in the mononuclear bone marrow cell fraction, indicating the induction of an immune response. Twelve patients received additional DLI dose escalation from 6 months after SCT. With a median follow up for survivors of 3.2 years (range 0.5-3.8), overall survival of these 20 high risk patients was 78% at 1 year and 65% at 3 years. Four patients died from relapse, 2 from infections.

In conclusion, administration of very low dose DLI early after TCD-SCT appears to be safe and effective, and allows further DLI dose escalation for disease control.

P1039

Extracorporeal photopheresis as cell therapy for lung transplant rejection

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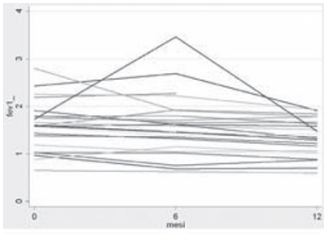
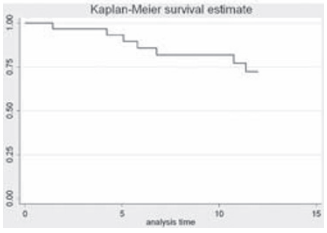
Objective: Lung transplanted pts have a high incidence of chronic rejection (bronchiolitis obliterans syndrome, BOS) notwithstanding an important immunosuppressive therapy (IST). BOS is an irreversible condition responsible for disappointing long term survival. IST have relevant side effects, mainly higher risk of infections and multiorgan damage. Extracorporeal photochemotherapy (ECP) is an immunomodulating cell therapy, with few side effects.It is essentially based on mononuclear cell collection, their UV-A irradiation in presence of 8-methoxypsoralen and reinfusion of the cells to the pts. The ECP mechanism of action is not completely understood but it probably involves leukocyte apoptosis, change in dendritic cell generation, production of cytokines and T reg induction. ECP has recently been employed in lung transplant rejection with the aim to stabilize graft function (calculated as the decline of forced expiratory volume in 1 second - FEV1). We report our experience on 37 pts treated by ECP from 2003 to 2010 with different grade of BOS for a progressive lung function decline not responsive to standard IST.

Methods: Over 37 pts with BOS, 32 were evaluable: 16 BOSI, 7 BOSII, 9 BOSIII. All pts were maintained under IST during ECP treatment. We retrospectively evaluated the ECP safety and efficacy (defined as decline of FEV1≤10% -stable- or improvement of FEV1 if compared to FEV1 at starting ECP), and median survival after ECP initiation.

Results: The total number of procedures was 1001 (mean 31.3/pts, range: 8-135). After 6 months of treatment 23 pts were evaluable, 17/23 responded (73.9%): 5 improved FEV1 and 12 were stable. Among responders 9/12 had BOS I, 3/5 BOS II, 5/6 BOS III. After 12 months, 21 pts were evaluable, 13/21responded (61.9%): all stable. 6/10 had BOSI, 3/5 had BOS II, 5/6had BOS III. Interestingly responders included 4 pts classified as not responders at six months. After 1 year, 23/32 pts were alive (71.9%). No relevant side effects during and after ECP procedures were observed. No increase in infection rate and transfusion demand were registered.

Conclusion: ECP confirmed to be a safe and effective therapy for lung transplanted pts not responsive to IST, independently from BOS grade. It stabilized the decline of lung function in a good percentage of patients. The optimal ECP schedule must be defined, considering that some patients show a late response (over 6 months). Our data need to be confirmed in a larger cohort of patients.





P1040

Bone marrow blood administration to patients with critical limb ischaemia

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Objectives: Promising reports about clinical improvement of critical limb ischaemia after autologous bone marrow blood (BMB) administration have prompted us to work with this approach. The aim of our study is to compare intramuscular (i.m.) and intraarterial (i.a.) BMB application as the question of the way of graft administration is currently unresolved.

Methods: In thirty-one patients (age 66±10 years, M:F 27:4) with advanced CLI (Rutherford class 5,0±0,2; transcutaneous oxygen pressure tcpO2=14±9 mmHg), that has failed or were not indicated for revascularization, we used 40 ml of concentrated autologous bone marrow blood with average total

nucleated cell number 4,4±1,2x10^9 (CD34+ cells 28±19x10^6). According to the randomization BMB was administrated i.m. (16 patients) into the ischemic limb or by means of i.a. infusion (800ml/hour) through the catheter positioned into the popliteal artery (15 patients). In follow-up we evaluated limb salvage, wound healing, delta tcpO2, quality of life (EQ 5D), and pain scoring (0-10). Criteria of clinical response were limb salvage and/ or wound healing.
Results: In 90 days follow-up from BMB administration clinical response was in 21 (79%) patients (including 6 high amputation). Two patients died from unrelated reason to study procedure. There was significant improvement in tcpO2 (14±9 to 35±18mmHg; p<0,001), pain scoring (4,1±2,7 to 0,8±1,3; p<0.001), EQ 5D (48±14 to 67±13; p<0.01), and significant decrease in Rutherford class (5,0±0,2 to 4,5±1,4; p<0.05) in the study group. There was significantly higher CD 34+ cell amount and lower serum C-reactive protein (CRP) in responders (21 patients) versus non-responders (8 patients): CD34+ cells 32,3±21x10^6 vs 19±13,4x10^6 (p<0,05); CRP 19±31 vs 72±81 mg/l (p<0,05). There was no difference in i.m. versus i.a. application in study parameters.
Conclusion: Autologous bone marrow blood administration is safe and there is no difference in intraarterial versus intramuscular application in advanced critical limb ischaemia. Higher CD34+ cell content in BMB and lower serum CRP are associated with good response.

P1041

Multiple myeloma and microenvironment formation: the role of CXCR4/CXCL12 chemokine pathway

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Multiple myeloma (MM) is characterized by clonal proliferation of malignant plasma cells (PCs) in the bone marrow (BM) compartment. Novel agents lenalidomide and bortezomib pre- and post allogeneic stem cell transplantation (SCT) as well as immunological based therapies significantly improved progression-free survival and overall survival of MM patients. Novel approaches are in need. We hypothesized that elucidating the role for CXCL12 chemokine and its receptor CXCR4 in MM-stroma interactions and microenvironment formation may have novel therapeutic applications. We now show that MM cell lines ARH77 and RPMI8226 and primary MM cells may produce high amounts of CXCL12 and co-express CXCR4 receptor. Co-culture of the MM cells with BMSCs significantly up-regulated both CXCR4 cell-surface expression and CXCL12 secretion by the MM cells. We found that BMSC-induced increase in CXCR4 and CXCL12 expression by MM cells was cell-contact dependent. Conditioned medium (CM) produced by MM cells cultured with BMSCs specifically attracted increased numbers of PB CD14+ cells in a CXCR4-dependent manner. Moreover, PB-generated macrophages induced the proliferation of MM cells, even more effectively than BMSCs. Furthermore, co-culture with macrophages strongly increased the expression of various pro-inflammatory and pro-angiogenic factors by MM cells, including CCL2, CCL4, IL-1b, IL-8 and VEGF. Interestingly, expression of IL-10 by MM cells was also up-regulated following the interaction with macrophages, suggesting the possible reciprocal effect of MM-produced factors on macrophage phenotype polarization. Our findings demonstrate that interaction of MM with BMSCs positively regulates the expression of CXCR4 and CXCL12 by MM cells, affecting both MM proliferation and CXCR4-dependent monocyte recruitment. Migrated monocytes may in turn interact with MM cells, support their growth and activate cytokine release, therefore producing favorable pro-inflammatory and pro-angiogenic environment and promoting disease progression. Our data provide the basis for future targeting MM-BMSCs and MM-macrophage interactions with anti-CXCR4 agents as therapeutic strategy in MM. However, further characterization of MM-microenvironment reciprocal interactions and deeper understanding of cellular and molecular mechanisms underlying these processes will enable identification of specific therapeutic targets which may be of benefit improving results of post transplantation anti MM therapies.

P1042

Bortezomib+mesenchymal sStem cells are a potent combination to treat experimental arthritis

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The role of Mesenchymal Stem Cells (MSCs) in animal models of rheumatoid arthritis (RA) has been an issue of debate due to contradictory reports suggesting either no effect or disease amelioration. In our hands, MSCs were ineffective in adjuvant-induced arthritis [(AIA),unpublished data] whereas the proteasome inhibitor, Bortezomib (Bzb), significantly ameliorated AIA by reducing inflammation and bone loss (Yannaki E, Arthritis Rheum 2010). We explored MSCs both in AIA and in spontaneous arthritis (SA, KRN model), in order to exclude the possibility that, in AIA, the injected FCA [containing Toll-like Receptor (TLR) ligands] is responsible for the in vivo inefficiency of MSCs by potentially polarizing them towards a proinflammatory phenotype. We aimed also to develop a strategy to circumvent the in vivo limitations of MSCs by combining them with Bzb-pretreatment in an effort to attenuate the inflammatory arthritic milieu before MSCs. Bone marrow MSCs were administered at established disease (days 21,24). A short Bzb-course was initiated at the onset of arthritis (days 13,16,19). Animals were sacrificed on day29. Tested groups were: control, MSCs-only, Bzb-only and Bzb+MSCs. MSCs or MSC supernatant inhibited the proliferation of fibroblast-like synoviocytes (FLS) and splenocytes (SPLCs) [p≤0.0009] and the FLS invasiveness. Despite the strong in vitro effect of MSCs, AIA and SA animals treated with MSCs-only weren't improved as compared to control ones. In contrast, Bzb+MSCs significantly decreased arthritis score over AA-, MSCs-only-, Bzb-only-treated animals (AIA:3.7±1.01 vs 8.9±1.02 vs 10.8±0.3 vs 8.4±0.9, p<0.0009/ SA:3.67±2.03 vs 8.4±0.9 vs 7.43±0.54 vs 8.0±0.7, p=0.001). The clinical improvement in the Bzb-only vs control group was significant on day21 (p=0.003) but transient as arthritis didn't differ from the control group at day of sacrifice. Histopathology and immunohistochemistry (CD3, FVIII, IL-6) findings directly correlated with the clinical score. The Bzb+MSCs combination altered the cytokine secretion pattern in the SPLC sup and restored the proliferation and apoptosis of SPLCs and the expression of TLRs (2,3,4) in treated animals as compared to arthritic ones. Our data suggest that the early administration of a short Bzb-course allows MSCs to retain their immunomodulatory function and act therapeutically in experimental arthritis and that the Bzb+MSCs combination may serve as a new therapeutic approach for RA.

P1043

Immunomodulation by escalated lymphodepletion and donor lymphocyte infusion to treat post-allogeneic transplantation relapse

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Lymphodepletion (LD) may enhance anti-tumor effects of transferred T cells. To prevent severe GVHD, we sequentially escalated both the LD and T cell doses. LD consisted of Fludarabine (Flu) 25 mg/m²/d for 3 d followed by infusion of 1x10^7 CD3+/

kg. If no GVHD developed, the same LD was followed by a 2d DLI of 5x10^7 CD3+/kg. In the next stage, LD combined both cyclophosphamide (Cy) 600 mg/m² for 1 d and Flu 25 mg/m²/d for 3 d followed by DLI at 5x10^7 CD3+/kg. Eighteen pts (45 y; 18-67) were treated for relapse (6 AML/MDS, 5 ALL, 3 HD, 2 T-NHL, 2 MM; 6 myeloablat, 12 RIC; 10 sib, 8 MUD). Forty four DLIs were performed including 28 LD-DLI. Ten, 6 and 2 pts received respectively 1, 2 or 3 LD-DLI. The 1st cohort (n=5) consisted of pts receiving LD with Flu alone. No severe toxicity was observed. In the 2d cohort (n=4) following a first LD-DLI with Flu alone, Cy was introduced along with Flu prior to DLI. Two pts developed aGVHD: 1 cut. gr 2 and another cut. gr 3, liver gr 3 and gut gr 4. The 3rd cohort included 5 pts who had received prior DLI without LD and who were then treated with LD with Flu/Cy followed by high dose (5x10^7 CD3+/kg.) DLI. Only 1 pt developed cut gr. 3 GVHD. In the 4th group, 4 pts who had not yet received DLI, received LD with Flu/Cy followed by high dose DLI. In this latter group, 1 case of aGVHD gr 4 was observed. The cumulative incidence of gr 2-4 aGVHD was 29.4 % with a median onset following the last LD-DLI of 31 (15-59) d. Twelve pts received cytoreduction chemotherapy prior to DLI. In 9 pts, this failed to result in a CR, leaving 3 CR after chemotherapy. Following LD-DLI, 4 additional pts achieved a CR. After a median follow-up of 8 months (2–28.3) from the first LD-DLI, 8 pts are alive; only 2 remain in CR. Ten pts died, 9 due to progression and 1 from lung cancer. OS from the 1st LD-DLI is 43.2 % (95%CI,20.3–66.1%) at 2 y. The absolute nbr of WBC, CD4+CD27+, CD8+, CD4+CD25high cells were compared prior to LD-DLI and 14 d following DLI, in those receiving Flu/Cy, and were found to be decreased, while the nbr of myeloid and plasmacytoid dendritic cells increased, none significantly. IL-7, IL-15 plasma levels remained undetectable at d14 post DLI. LD-DLI in an escalated manner is not complicated by excessively severe GVHD while transient remission can be achieved. In our pts, this did not translated into significant modification of T subsets or expansion of homeostatic cytokines. Further studies with escalating doses are planned.

P1044

Double positive and double negative TCR γ delta+ cells independently generate mature functional TCR γ delta + cells in the human thymus

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Background: Immunotherapy studies mainly focus on induction of a TCRα-β mediated T cell response. Recent studies however show that also TCRγ-delta+ cells play an important role in tumor control. A major advantage of TCRγ-delta+ cells is that they mediate an HLA-unrestricted highly cytotoxic activity against most tumor cell types. A drawback of TCRγ-delta+ cells is that it is still unclear how these cells are generated in human. Mouse studies show that TCRγ-delta+ cells become mature effector cells without ever passing through the CD4+ CD8+ DP stage, as is the case for TCRα-β+ cells. In human, however, a prominent immature TCRγ-delta+ DP population is observed in postnatal thymus (PNT). In this study, we investigated the role of the DP immature TCRγ-delta+ population.
Methodology: Immature TCRγ-delta+ DN and DP cells were sorted from PNT and precursor-progeny relationship was studied in vitro. TCR rearrangements were studies and functionality of mature cells was tested.
Results: We found that immature TCRγ-delta+ DN cells differentiate into mature, functional TCRγ-delta+ DN or CD8αα SP cells without passing through the DP stage. Differentiation was Notch independent but IL-7 dependent. In the presence of Notch stimuli and IL-7, a population of immature TCRγ-delta+ DP arises. These DP cells rearrange the TCRα locus with loss of TCRγ-delta expression and generation of a small population of CD3+ TCRα-β+ DP cells. DP cells that retain TCRγ-delta expression give rise to mature, functional TCRγ-delta+ CD8αα and CD8α-β SP cells. Immature TCRγ-delta+ DP and mature TCRγ-delta+ CD8α-β SP cells contain TCRα rearrangements, suggesting that also in vivo mature CD8α-β SP TCRγ-delta+ cells are derived from TCRγ-delta+ DP cells.
Conclusion: We show that human TCRγ-delta+ cells differentiate along 2 pathways: a DN pathway that generates mature DN and CD8α-α SP TCRγ-delta+ cells and a DP pathway that generates mature CD8α-α and CD8α-β SP TCRγ-delta+ cells.

P1045

Ex vivo generation of donor-derived WT1-specific cytotoxic T-cells able to lyse patient's leukaemia blasts for adoptive immunotherapy after allogeneic haematopoietic stem cell transplantation

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The Wilms tumor antigen, WT1, has been described as a key molecule for tumor proliferation in a large number of human malignancies. Over expression of WT1 has been documented in various types of leukemia and solid tumors. In this study, we investigated the feasibility of in vitro generating and expanding WT1 peptide-specific T cells of donor origin and investigated their capacity to lyse patient's leukemia blasts (LB), expressing WT1, as a prerequisite for adoptive T-cell therapy after allogeneic hematopoietic stem cell transplantation (HSCT). Using a slightly modified methodology, previously employed for the induction of anti-leukemia CTLs directed against whole tumor cells (LB-CTLs), we evaluated the possibility of generating WT1-specific CTLs in 5 donor/recipients pairs starting from peripheral blood (PB) of HLA-A2+ HSC donors. CTLs were generated starting either from PBMC or CD8-enriched lymphocytes stimulated with DC pulsed with WT1 peptides in the presence of IL-7 and IL-12. Cells were restimulated in the presence of irradiated donor mononuclear cells pulsed with WT1-peptides and expanded in an antigen independent way. CTLs displayed high levels of cytotoxicity against WT1-pulsed donor PHA-blasts, media 50% ± 8% at effector/target (E:T) 25:1, and negligible levels (<10% lysis at E:T ratio of 25:1) against donor PHA-blasts pulsed with irrelevant peptides or with medium alone. WT1-specific CTLs from 4 out 5 donors displayed sizeable levels of cytotoxicity against patients' LB (media 37% ± 13% at E:T ratio of 25:1). Patients' LB that were not lysed by donor-derived WT1-specific CTLs expressed lower levels of WT1 compared to other primary blasts.
These data confirm the possibility of obtaining a large quantity of donor-derived WT1-specific CTLs, able to lyse patients' LB, from PB of HSCT donors, and suggest the possibility of utilizing these in adoptive immunotherapy to control/prevent leukemia relapse in HLA-A2 patients given allogeneic HSCT. WT1-specific CTL infusions could represent an alternative approach in case of an insufficient number of primary LB to generate LB-CTLs, or be used together with LB-CTLs, directed against the whole leukemia cells, to improve their efficacy.
Further experiments are in progress to evaluate the possibility of generating WT1-specific CTLs from more donor/recipient pairs and their phenotypical and functional characteristics and compare levels of lysis against LB and WT1 expression levels on leukemia samples.

P1046

Risk factors for non-relapse mortality in 287 patients receiving 1288 donor lymphocyte infusions

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Aim of the study: To assess the risk of graft versus host disease (GvHD) and non relapse mortality (NRM).
Patients: We analyzed 287 patients who received a total of 1288 DLI, for different reasons and at different intervals from transplant. The median number of DLI was 4 (1-15) the median interval between transplant and DLi was 282 dasy (1-4480), the median number of infused CD3 cells/kg of recipients body weight was $1x10^7$ ($1x10^3$-$5x10^7$). The diagnoses were chronic myeloid leukemia (n=78), acute leukemia (n=100), myelofibrosis (n=21), myelodisplastic syndrome (n=14), lymphoma (n=35), other diagnosis (n=38). Median patients age was 40 (12-68).
Statistical analysis: Factors studied for an association with GvHD and NRM were donor type (siblings/alternative donors), diagnosis (CML others), year of DLI (</> 2001), maximum dose of DLI (</> $1x10^7$), recipient age (≤/>40 years), donor age (≤/> 40 years), number of DLI (</> 4), interval transplant-DLI (</> 282 days), phase of the disease at transplant (early/advanced), and gender (donor recipient), reason for DLI (relapse/non relapse).
Results GvHD: Seventy patients (24%) developed acute GvHD grade II-IV. In univariate analysis we coujld only identify the year of transplant as a predictor: GvHD II-IV developed in 29% of patients grafted before 2001 and in 19% of patients grafted later (p=0.04). In multivariate analysis this result was confirmed.
Results NRM: With a median follow up for surviving patients of 2346 days (125-7194) 164 patients survive (57%). The primary cause of death was relapse of the original disease in 97 patients (34%), whereas 26 died of NRM (8%).
Factors predicting NRM in univariate analysis were interval diagnosis-DLI, donor type and patient age. In multivariate COX analysis, the strongest predictor for NRM was the interval between transplant and DLI (p=00001) with a RR of 6.6 of NMR for patients receiving DLI before the median intgerval (282 days). Other predictors were donor type (siblings had a risk of 0.43, p=0.01), disease phase (patients with early disease had a RR of 0.3) and DLI for reasons other than relapse (RR 3.6).
Conclusions: In this relatively large series of consecutive DLI, the risk of acute GvHD was relatively low, and we could not identify significant predictors. NRM was strongly associated with the interval between transplant and DLI, and this should be considered when councelling patients before DLI early after transplant.

P1047

Antileukaemic T-cell responses after DC-stimulation can be predicted by composition of T-cell subpopulations especially with respect to naïve, central memory and non-naïve T-cells

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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 effectual in every case.
Methods: The aim of our study was to further enlighten the role of the composition of T-cell subpopulations (Naïve (Tnaive), non-naïve (Tnon-naïve), effector (Teff), effector memory (Tem) and central memory (Tcm)) for the mediation of antileukemic reactions.
In 0-7 days' mixed lymphocyte cultures (MLC) we stimulated autologous (n=6) or allogeneic T-cells (n=1) or T-cells after SCT (n=5) with DC or blasts and studied their antileukemic reactivity compared to unstimulated T-cells in a functional Fluorolysis assay.
Results: Uncultured cells presented with on average (Ø)14% Tnaive, Ø68% Tnon-naïve, Ø15% Teff, Ø32% Tem and Ø19% Tcm. After Blast- and even more after DC-stimulation in an MLC Tnon-naïve as well as Teff significantly increased to Ø73%/37% (p=0.002) whereas Tnaive/ Tcm/ Tem decreased to Ø4% (p=0.001)/13%/21%(p=0.08). Antileukemic functionality was achieved in 7 of 11 (64%) cases after DC-stimulation compared to 4 of 10 cases (40%) after blast stimulation. Cases with/without lytic activity after DC- (but not after blast-)stimulation were characterized by Ø4/1% Tnaive, 25/13% Tem, 12/3% (p=0.02) Tcm and 75/56% Tnon-naïve. Interestingly cases with/without lytic activity after DC-stimulation presented with 15/5% (p=0.07) Tnaive and 13/25% Teff and 60/78% (p=0.09) Tnon-naïve before DC-stimulation. Kinetic studies showed that the T-cellular profiles developed during the first 5 days of DC/blast stimulation. After DC (but not after blast) stimulation 100/100/86% of cases showed antileukemic T-cell activity, if 2.5/11/59% Tnaive/ Tcm/ Tnon-naïve were found after MLC. All results obtained were comparable in allogeneic, autologous settings or using T-cells after SCT.
Conclusion: Our data confirming the central role of DC in the mediation of antileukemic T-cell response: Cases with lytic activity are characterized by higher proportions of Tnaive, Tcm and Tnon-naïve. This could contribute to define values to predict antileukemic responses. Moreover this could indicate the hypothetic creation of a central antileukemic memory and effector cells induced through DC, but not through blast stimulation.

P1048

Antileukaemic T-cell responses after dendritic cell stimulation can be predicted by compositions of regulatory T-cell subpopulations, especially with respect to regulatory central memory and regulatory CD8 cells

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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 regulatory T-cell (Treg) subpopulations: Naïve (Tnaive reg), Central memory (Tcm reg) or Effector(memory) Treg (Teff/emm reg) for the mediation of antileukemic reactions. In 0-7 days' mixed lymphocyte cultures (MLC) we stimulated autologous (n=6) or allogeneic T-cells (n=1) or T-cells after SCT (n=5) with DC or blasts and studied their antileukemic reactivity compared to unstimulated T-cells in a functional Fluorolysis assay.
Results: Uncultured cells presented with on average (Ø)31% of CD4+ Treg in CD4+, Ø14% of CD8+ Treg in CD8+, Ø32% Tnaive reg in all Tnaive, Ø34% of Tcm reg in all Tcm and Ø19% of Teff/em reg in all Teff/em cells. After Blast and DC stimulation in MLC CD4+ Treg and CD8+ Treg increased to Ø49(p<0.01)/53%(p<0.001), Tnaive reg to Ø78%, Tcm reg to Ø57% and Teff/em reg to Ø58%. Antileukemic functionality was achieved

in 7 of 11 (64%) cases after DC-stimulation compared to 4 of 10 cases (40%) after blast stimulation. Cases with/without lytic activity after DC- (but not after blast) stimulation were characterized by Ø51/50% CD4+ Treg, Ø44/76% (p<0.01) CD8+ Treg, Ø75/85% Tnaive reg, Ø54/70% Tcm reg and Ø49/75% (p=0.02) Teff/em reg. These functional differences could not be shown after blast stimulation. Kinetic studies showed, that the T-cellular profiles developed during the first 3 days of stimulation. After DC (but not after blast) stimulation 100% of cases showed antileukemic T-cell activity, if <60% CD8+ Treg or <60% Teff/em reg were found after MLC. All results were comparable in allogeneic, autologous settings or using T-cells after SCT.
Conclusion: Our data confirm the central role of DC in the mediation of antileukemic T-cell response: Cases with lytic activity present with lower proportions of CD8+ Treg, Teff/em reg and Tcm reg. This helps to define values to predict antileukemic responses and criteria to single out cases by potential exclusion criteria in which stimulation with DC will not lead to antileukemic activity, e.g in the context of DC vaccinations.

P1049

Effect of allogeneic bone marrow mesenchymal stem cells on proliferation and apoptosis of normal haematopoietic progenitors and leucaemic cells

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Objectives: The use of transplantation of mesenchymal stem cells (MSCs) to accelerate the engraftment of hematopoietic precursors and for the correction of graft-versus-host disease (GvHD) in malignant hematological diseases makes one wonder what influence may have MSC on leukemic cells present in bone marrow of the recipient in the form of minimal residual disease. The aim: investigate the mechanism of the effect of allogeneic bone marrow MSCs on the ability of tumor cells to spontaneous and induced apoptosis.
Methods: Leukemic cells from bone marrow samples of patients (age 6 months - 17 years) with newly diagnosed acute leukemia (AL): B-ALL-41, T-ALL-12, AML-18. MSC (from 43 healthy donors) were cultured in DMEM with 20% FCS. Clonogenic study of granulocyte-macrophage bone marrow progenitors (from 15 healthy donors) were performed in semisolid agar medium. The sensitivity of blast cells to cytotoxic drugs was studied by the MTT-assay. To determine the levels of spontaneous and induced apoptosis in leukemia cells we use Apoptosis Detection Kit II (BD). Cytokines produced by MSCs was determined by flow Cytofluorometer using reagents BD Cytometric Bead Array.
Results: MSCs stimulate colony formation of granulocyte-macrophage precursors, surpassing the effectiveness of phytohemagglutinin-leukocyte conditioned medium as a source of colony stimulating factors. Efficiency of cloning was 27.9 (SD 1.5) and 22.9 (SD 2.4), respectively. When leukemic cells were cultured on MSC for 4 days the proportion of viable cells was higher at 47.2% in ALL, and 63.1% in AML compared with controls. Incubation of leukemic cells with MSCs resulted in a decrease of sensitivity to cytarabine in 2 times in B-ALL, in 1.5 times in AML and 6 times in T-ALL. Under the influence of MSCs sensitivity of leukemic cells to daunorubicin decreased in all groups. MSC increased the sensitivity of blast cells from ALL patients to MP, but did not influence the sensitivity of AML blast cells to MP. MSCs inhibit apoptosis of leukemic cells induced by cytarabine. The study of MSCs cultures with SMA showed that MSCs produce a wide range of cytokines (IL-2, 4, 6, 10; FGF, VEGF), which may mediate the effect of MSCs on the viability of hematopoietic progenitors and leukemic cells.
Conclusion: On the basis of the research is clear that the important aspect is the safety of MSCs in terms of their influence on the sensitivity of leukemic cells to chemotherapy in patients with hematologic malignancies.

P1050

Analysis of chimerism of bone marrow mesenchymal stem cells in patients undergoing allogenic haematopoietic stem cell transplantation

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Objective: Whether human mesenchymal stem cells (MSC) can be transplanted is controversial. In this report we studied Chimerism status of bone marrow (BM)-derived mesenchymal stem cells (MSC) from children who received allogenic hematopoietic transplantation using BM, peripheral blood (PB), and umbilical cord blood (UCB) as a source of hematopoietic stem cells (HSC).
Methods: Bone marrow MSC from 28 patients (ALL-8, AML-20), who received allogenic transplantation were expanded up to third-fourth passage. MSCs was identified using immunophenotypic markers (positive for CD44, 73, 90,105 and negative for CD14,34,45). After that, chimerism studies were performed using reverse transcription polymerase chain reaction of short tandem repeat (STR) loci. Analyses were carried out at different time-points after transplantation (+22 - +302 days), with a total of 64 samples studied. BM was used as the source of stem cells for transplantation in 16 cases, PB in 11 cases and UCB in 1 case. The conditioning regimen was standard in 19 patients and non-myeloablative in 9 patients.
Results: After cultivation, in the great majority of cases (26 out 28; 93%) MSC were of host origin, despite complete chimerism in BM and PB. However, in 2 patients with complete (1 with ALL after transplantation using PB stem cells and 1 with AML after BM transplantation) BM-derived MSC were of recipient origin. Donor chimerism of the first patient was 27% at day 52 and dramatically decreased to 6% at day 58. MSC chimerism of the second child was 24% at day 33 and more gradually reduced to 17% at day 81. Further samples of BM MSC from both children showed MSC chimerism of the recipients.
Conclusions: Our method for determining MSC chimerism by STR loci was quite informative and can be used to assess the engraftment of MSC in recipients after allogenic transplantation of HCS. This study indicates that after allogenic transplantation MSC from the donor can engraft in BM. Moreover, since in 1 case the stem cells were obtained from PB, it can be concluded that MSC circulate among mobilized PB stem cells and can engraft in BM after allogenic transplantation.

P1051

Cytotoxic capability of IL15 stimulated CIK cells in humanized NOD/SCID/IL2Rγ(null) mice transplanted with rhabdomyosarcoma and acute myeloid leukaemia cells

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Objectives: Haploidentical stem cell transplantation (HSCT) has become an important treatment modality for children and adolescents with high-risk leukemia and is also more frequently used as a therapeutic tool for patients with solid malignancies such as rhabdomyosarcoma (RMS). Cytokine-induced killer (CIK) cells may serve as an alternative approach to adoptive donor lymphocyte infusions (DLI) for patients to prevent relapse after HSCT.
Methods: CIK cells were activated using IL-2, IL-15 or cytokine combination. Cytotoxic capacity of CIK cells was evaluated in vitro and in vivo in humanized NOD/SCID/IL2Rγ (null) mice transplanted with RMS and acute myeloid leukemia (AML) cell lines. In vitro cytotoxicity of CIK cells was determined by europium release assay. Engraftment and expansion of AML and RMS cells in mice were monitored by FACS, PCR-based detection of human albumin and chimerism analysis.

Results: IL-15 stimulation significantly enhanced cytolytic capacity of CIK cells against RMS (specific lysis: TE671, 3.0 ± 5.2% vs 46.0 ± 19.6%, E:T ratio, 4:1; RH30 3.5 ± 4.1% vs 27.8 ± 7.9%, E:T ratio, 4:1) and leukemia cell lines (specific lysis: MOLT4, 4.8 ± 6.6% vs 48.5 ± 11.5%, E:T ratio, 13:1; THP-1, 24.7 ± 7.4% vs 46.4 ± 13.6%, E:T ratio, 6:1) in vitro. Cytotoxic capacity of IL-15 activated CIK cells was subsequently evaluated in mice injected with human RMS and AML cells. Substantial anti-tumor (3-fold reduction of RH41 cells, 202.5 vs 66.9 relative fluorescence units (RFU), E:T ratio, 1:1) and anti-leukemic activity (5-fold reduction of THP-1 cells, 7.2 vs 1.4 of total CD45+CD33+ cells, E:T ratio, 1:1000) was observed in vivo. Moreover, no side effects were detected in mice transplanted with IL-15 stimulated CIK cells.
Conclusion: In conclusion, these findings provide first evidence that IL-15 expanded CIK cells may offer an improved pre-emptive immunotherapeutic approach for patients suffering from leukemia or solid malignancies facing impending relapse after HSCT.

P1052
Molecular remission of FLT3-ITD+ positive AML relapse after allogeneic stem cell transplantation by acute GvHD in addition to sorafenib
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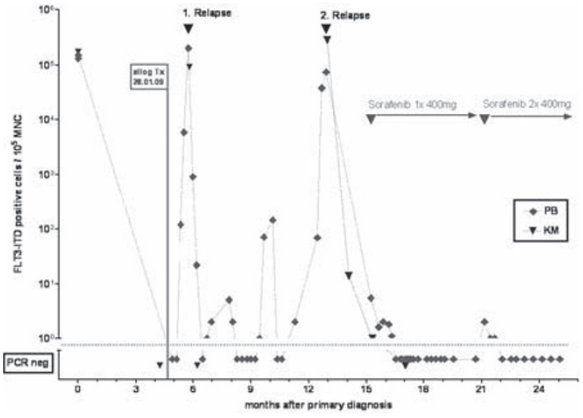
Mutations of the FLT-3 gene worsen the prognosis in AML, however, they provide a target for a new therapeutic approach with sorafenib.
A 57 year old male suffering from AML with an FLT3-ITD and a mutated NPM gene proceeded to allo-SCT after two cycles of idarubicin+HD-Ara-C. BM smear showed CR after the 1st induction cycle. Mutations were detected by PCR. The patient was conditioned with TBI (2Gy), fludarabine 150 mg/m² and ATG and was grafted with 12.3*10e6 CD34+ cells from an unrelated donor. GvHD-prophylaxis was carried out with CsA, MMF and MTX. Neither a significant leukopenia (<1.0/nl) nor thrombocytes <20/nl were observed. Donor chimerism in BM was 100% at day +28. The FLT3-ITD mutation in AML blasts was sequenced and a clone specific quantitative rt-PCR with a sensitivity of 10-5 to -6 was established. Relapse of AML was diagnosed few weeks later and immunosuppression was discontinued. At day +35 aGvHD of the skin (III°) occurred and was treated with steroids and CsA. BM puncture at day +47 revealed again a CR of AML with 100% donor chimerism. Extracorporal photopheresis was initiated against cGvHD. A 2nd relapse of AML was detected 8 months after allo-SCT and ECP was discontinued. A 3rd remission was achieved with one course of idarubicine+Ara-C and was consolidated with another cycle. Sorafenib (400 mg/d p.o.) was initiated after recovery with full donor chimerism and cGvHD (extensive disease) reoccurred. Initially, FLT3-ITD+ copy numbers could be reduced by >5 log steps by chemotherapy and after 1st relapse by transplant-induced GvL-effects. However, the 3rd molecular remission in blood and marrow was finally achieved by sorafenib in conjunction with GvL-effects as indicated by relapse of chronic GvHD. Actually, the AML is in 3rd CCR after SCT for 8 months. The patient is doing well (ECOG 0) 22 months after allo-SCT and cGvHD is restricted to the oral cavity.
The presented case shows that sorafenib in conjunction with GvL-effects has the power to improve a clinical into a molecular remission after 2nd relapse a FLT3+ AML. It could be argued that it is impossible to discriminate the contribution of both compounds, however, the follow-up of minimal residual disease in blood and marrow suggests a synergy of both effects. Presently, cGvHD is well controlled and the patient is still MRD-negative at a sensitivity of 10-5 in the presence of oral therapy with 2x400 mg sorafenib.



P1053
Improving the function of TCR gene modified T-cells for adoptive immunotherapy: co-transfer of additional CD3 molecules enhances functional avidity of TCR-transduced CD4+ T-cells
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To date the majority of TCR gene therapy studies have focused on transfer of TCR genes into CD8+ T cells in order to generate large numbers of antigen-specific T cells for adoptive immunotherapy. There is evidence that the transfer of antigen specific CD8+ T cells in the absence of antigen specific CD4+ T cells impairs anti-tumor response and memory development in vivo. Previous work has explored the transduction of class I restricted F5-TCR (specific for influenza peptide NP in the context of H2-Db class I molecules) into murine CD4+ T cells. F5 TCR-transduced (Td) CD4+ cells produced cytokines in response to specific antigen and provided in vivo help for tumor protection in murine models. The F5 TCR-Td CD4+ T cells were partially CD8+ dependent: F5 TCR-Td CD4+ T cells are functional but require 10 fold higher peptide concentrations for antigen recognition compared to F5 TCR-Td CD8+ T cells. In order to improve the function of class I restricted TCR expressing CD4 T cells we have co-transduced additional CD3 molecules as TCR-CD3 complex assembly is rate limiting for surface expression of introduced TCR. We hypothesised that increased TCR expression would result in increased functional avidity.
Using a retroviral transduction protocol, CD4+ T cells transduced with F5-TCR and CD4+ T cells transduced with F5-TCR and CD3-GFP were generated. Functional assays were performed (ELISA for cytokine production, proliferation assay) on transduced T cells following overnight stimulation with irradiated splenocytes pulsed with relevant or irrelevant peptide. CD4+T cells transduced with F5 and CD3 have increased surface expression of TCR when compared to cells transduced with F5 TCR alone. Using ELISA to measure IL2 and IFN-g production, CD4+ T cells co-transduced with F5 TCR and CD3 were of higher avidity than those transduced with F5 alone and recognised 10-fold lower concentrations of relevant peptide than cells transduced with F5 TCR alone. F5 TCR-CD3-CD4+ T cells had higher levels of proliferation than F5 TCR-CD4+ T cells when stimulated with relevant peptide and were able to respond to 100-fold lower concentration of peptide. Our in vitro results suggest that co-transduction of CD3 and class I restricted TCR into CD4+ cells increases their functional avidity. In vivo adoptive transfer experiments looking at expansion and persistence in response to specific antigen and tumor protection utilizing TCR-CD3-Td-CD4+ T cells are ongoing.

P1054

Cytokine profile of patients with invasive aspergillosis: initial results from the first 100 patients recruited into the aspergillosis study; Great Britain

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Objectives: Invasive Aspergillosis (IA) is an important cause of morbidity and mortality in haemato-oncology patients undergoing haematopoietic stem cell transplantation (HSCT) or high-dose chemotherapy. We set up an observational prospective cohort study in order to improve our diagnostic and management strategies using the EORTC/MSG criteria as a diagnostic tool. Here we assess the serial cytokine profile of patients to evaluate their potential role in the diagnosis and management of IA.

Methods: All study patients were prospectively recruited and followed up for ≥ 4 months after chemotherapy or HSCT and had baseline and fortnightly follow-up serum samples profiled for 30 inflammatory cytokines using multiplex bead immunoassays by Luminex 100TM instrument. The cytokines measured were: EGF, Eotaxin, FGF, G-CSF, GM-CSF, HGF, IFN-a, IFN-g, IL-1RA, IL-1b, IL-2, IL-2R, IL-4, IL5, IL6, IL7, IL-8, IL-10, IL-12p40/p70, IL-13, IL-15, IL-17, IP-10, MCP-1, MIG, MIP-1a, MIP-1b, Rantes, TNF-a and VEGF. The first hundred patients enrolled with sufficient follow up data were included in this initial analysis. We used generalised logistic regression model for binary outcome of proven/probable and no evidence of IA (R2=0.4355, P<0.001) adjusting for clustering, age, sex, underlying diagnosis and treatment.

Results: The median (range) age was 52.5 (19-73) years and M/F ratio was 58/42. The main diagnosis were AML/MDS (40), Myeloma (23), NHL (17) and aplastic anaemia (9) treated by allogeneic HSCT (43), autologous HSCT (32), chemotherapy (20), and immunosuppressive therapy (5). The diagnosis of invasive fungal infection was based on the revised 2008 EORTC/MSG criteria. The incidence of proven and probable infection was 17% and possible infection accounted for 12%. Eight patients were excluded from analysis because of lack of proper baseline sample. Ten cytokines were found to be significantly different. Patients with IA were found to have lower IL-1b (P=0.003), IL-10 (P<0.001), IL-12 (P=0.05), IL-17 (P=0.016), IL-15 (P=0.027), INF-a (P=0.05), and GMCSF (P=0.023) but higher IL-6 (P=0.08) and IP-10 (P=0.022). Older age correlated with IA (P=0.014).

Conclusion: Overall patients with invasive fungal infection have significantly lower pro-inflammatory cytokine profile in the Th1 and Th17 axis and therefore unable to effectively deal with infection. If this profile is validated in the larger cohort it may be used as a predictive model for targeted anti-fungal prophylaxis.

P1055

Characterization of of regulatory T-cell subpopulations: phenotype, function and stability

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Regulatory T cells (Tregs) play a pivotal role in the induction of immune tolerance to alloantigens in hematopoietic stem cell transplantation (HSCT). Recent clinical results demonstrate the highly effective potency of Tregs to control graft-versus-host disease. However, current Treg isolation protocols only achieve a purity of approximately 50% CD4+CD25+FoxP3+ Tregs after depletion of B-cells and enrichment of CD25+ cells. Based on the identification of new Treg subpopulations and promising surface marker, we compared different Treg cell isolation strategies in order to define the most promising Treg target cell population for cellular intervention studies. For this purpose, isolated Treg populations have been analyzed for purity, function and stability of the suppressive phenotype: (I) CD4+ and CD25+ enrichment, (II) depletion of CD49d+ and CD127+ T cells, (III) enrichment of CD4+ CD25+ and depletion of CD127+ T cells and (IV) enrichment of CD4+ CD25+ CD45RA+ T cells. The highest purity of freshly isolated Tregs could be obtained for CD4+CD25+CD127- T cells (>99%; protocol III).

In contrast, after depletion of CD49d-CD127- cells the target cell fraction could only reach a purity of 80% for CD4+CD25+ Tregs. These Tregs exhibited the lowest Foxp3 expression before expansion, while the highest Foxp3 expression (57%) analyzed by FACS was measured in naïve CD4+CD25+CD45RA+ Tregs. After 14 days in vitro culture the Foxp3 expression in CD4+CD25+CD45RA+ Tregs decrease significantly (32%). Functional analysis revealed suppressive properties of every target cell population. However, the CD127+ depleted CD4+CD25+ Tregs were most suppressive (58% at a Treg/Tresp ratio of 1:1), while CD4+CD25+ enriched Tregs (protocol I) were less suppressive (32% at a Treg/Tresp ratio of 1:1). The stability of Tregs can be monitored by analysis of the Foxp3 TSDR demethylation status. In contrast to CD4+CD25+ Tregs (protocol I) with a stable Foxp3 phenotype, CD49dCD127- depleted Tregs (protocol II) showed the lowest Foxp3 TSDR demethylation levels. Nevertheless, these Treg cell fraction had good expansion properties. Whereas the isolation of CD4+CD25+CD127- Tregs seems to be the most promising approach for direct Treg cell transfer to the patients, CD4+CD25+CD45RA+ Tregs seem to be the most promising target cell fraction for in vitro expansion needed for the transfer of higher cell numbers or repetitive Treg cell infusions.

P1056

Influence of donor's and recipient's HPA-differences on duration of aplasia after allo-myelotransplantation

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It's known that platelets glycoproteins with HPA are located on CD34+ cells and consequently, on progenitor of megakaryocytes. The influence of HPA-distinctions on engraftment of HSC is not enough investigated. The purpose of the work - to study influence of the donor's and the recipient's HPA differences on duration of aplasia after HSC transplantation. A method of research - polymerase chain reaction with allele-specific primers (ASP-PSR) for identification of eight allele genes of loci HPA-1,-2,-3 and-5 (firm Protrans, Germany). DNA was allocated from bone marrow nuclei cells of 40 patients with oncohematological diseases before HSC transplantation and peripheral blood leukocytes of their HLA-identical siblings. Output from aplasia after transplantation estimated on achievement of neutrophil's count 0,5x109/l and platelet 50x109/l after stopping of platelet transfusions. Patients have been divided into groups - HPA-identical (1 group) and differing on HPA-genes: HPA-compatible (2 group) - the recipient had a distinguished genes in heterozygous, and the donor - in the homozygous variant, HPA-incompatible (3 group) - the recipient had distinguished HPA in homozygous, and the donor - in a heterozygous variant or they have allelic differences on whole HPA-locus. A median of netropenia duration at recipients of first two groups was 13,5 and 14,3 days, at recipients of 3 group - 19 days (p<0,001 and p=0,003, accordingly). The beginning of output from thrombocytopenia - 16 and 18 days at recipients of first two groups, and 24 days at recipients of 3 group (p=0,037 and p=0,012, accordingly). We assume, that incompatibility on HPA, located on platelet's glycoproteins, being adhesive molecules and integrins, can lead to change of the adhesive profile of progenitor cells with bone marrow's stromal cells ligands.

P1057

Leukaemia and epithelial cancer cells trigger NK cell storming: the NK cell alloperturbation phenomenon

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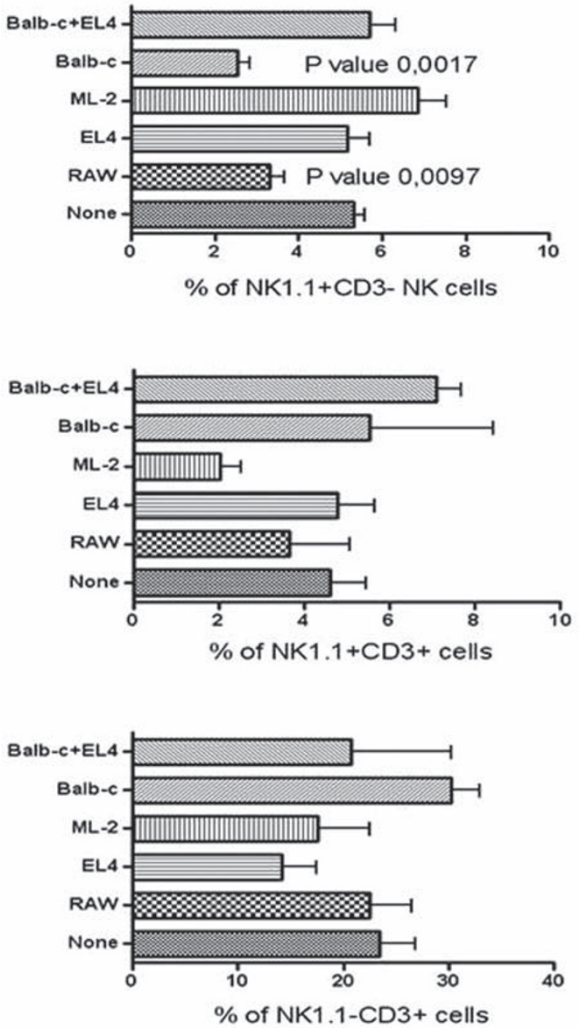
Background and rationale: Human NK/cancer cell interaction results in NK cell apoptosis and cancer cell killing. Although, natural cytotoxic receptors were involved in regulating NK cell apoptosis, the nature and specificity of such an interaction are still unknown.

Methods: Utilizing human leukemia (ML-2,THP-1,T2, and K562), human cancer (PANC-1, Caki-1, HCT-116, LS180, COLO-205, SW-480,D10,HBL and HeLa), mouse leukemia (RAW-264.7, H2d; EL4, H2b) and mouse melanoma (B16,H2b) cell lines, we investigated their effects on NK cells by flow cytometry and confocal microscopy.

Results: Human malignant cells (leukemia and epithelial) triggered perturbation of NK cells including (i) NK cell elimination; (ii) NK cell apoptosis; (iii) FcγRIII (CD16) down-regulation; and (iv) CD16 colocalization with HLA-A2 antigen on target cells at the immunological synapses. RAW-264.7 mouse leukemia cells induced C57BL/6J mouse NK cell elimination and to a lesser extent NK cell apoptosis. This perturbation involved human CD16+CD56+CD3-NK cells and mouse NK1.1+CD3- NK cells while human CD16-CD56+ cells, NK-T and mouse NK-T cells were not. Human soluble CD16 molecule bound unknown antigens expressed on human leukemia and epithelial cancer cells (ML-2,THP-1 and PANC-1) but did not bind mouse EL4 or allogeneic PBMC suggesting that CD16 recognized specific molecules widely expressed on human malignant cells. To evaluate the specificity of NK cell perturbation, we compared the effect of normal and leukemia cells including allogeneic BALB/c (H2d) splenocytes, allogeneic RAW-264.7(H2d),syngeneic EL4(H2b), syngeneic B16 (H2b), and xenogeneic ML-2 cells on C57BL/6J, NK1.1+CD3- NK cells. Figure 1 shows that RAW-264.7 and BALB/c splenocytes induced C56BL/6J, NK1.1+CD3- NK cells. However, BALB/c splenocytes pre-cultured with EL4 cells, failed to induce C57BL/6J, NK1.1+CD3- NK cell elimination suggesting that NK cell perturbation phenomenon requires specific recognition of cell surface molecules expressed on allogeneic malignant cells. This phenomenon was killer inhibitory receptors and caspase independent since total elimination of cell surface MHC-I expression on human and mouse tumor cell lines and ZVAD treatment did not affect NK cell alloperturbation respectively.

Conclusions: This study provides new insights on the nature and specificity of the NK/cancer cell interaction. It could also be of interest to anyone is involved in utilizing aploidentical NK cells immunotherapy of myeloid leukemia.



P1058

Clinical-grade generation of active NK cells from cord blood haematopoietic progenitor cells for immunotherapy using a closed-system culture process

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Natural killer (NK) cell-based adoptive immunotherapy is a promising treatment approach for many cancers. However, development of protocols that provide large numbers of functional NK cells produced under GMP conditions are required to facilitate clinical studies. In this study, we translated our cytokine-based culture protocol for ex vivo expansion of NK cells from umbilical cord blood (UCB) hematopoietic stem cells into a fully closed, large-scale, cell culture bioprocess. We optimized enrichment of CD34+ cells from cryopreserved UCB units using the CliniMACS system followed by efficient expansion for 14 days in gas-permeable cell culture bags. Thereafter, expanded CD34+ UCB cells could be reproducibly amplified and differentiated into CD56+CD3- NK cell products using bioreactors with a mean expansion of more than 2,000 fold and a purity of >90%. Moreover, expansion in the bioreactor yielded a clinically relevant dose of NK cells (mean: 2-4x10E9 NK cells), which display high expression of activating NK receptors and cytolytic activity against K562. Finally, we established a versatile closed washing procedure resulting in optimal reduction of medium, serum and cytokines used in the cell culture process without changes in phenotype and cytotoxic activity. Furthermore we performed extensive product release tests to provide a certificate of analysis for the NK cell product (see table). These results demonstrate that large numbers of UCB stem cell-derived NK cell products for adoptive immunotherapy can be produced in closed, large-scale bioreactors for the use in clinical trials.

[P1058]

Test	Method	Specification	Donor 10	Donor 13	Donor 15	Donor 16
NK cell number	FCM	$CD56^+CD3^-$ NK cells	$2.2x10^9$	$2.4x10^9$	$3.7x10^9$	$1.6x10^9$
Purity	FCM	>70% $CD56^+CD3^-$ NK cells	95%	90%	92%	92%
Viability	FCM	>70% 7-AAD negative	n.a.	98%	97%	93%
Phenotype	FCM	>30% positivity for CD56, CD94, NKG2A, NCR and NKG2D.	yes	yes	yes	yes
Karyotyping	Cell culture	Normal karyotype	yes	yes	yes	yes
Recovery	FCM	% $CD56^+CD3^-$ NK 7-AAD negative cells.	n.a.	83%	86%	76%
Content $CD3^+$ T-cells	FCM	< $1x10^4$ $CD3^+$ T cells/kg body weight of the patient	n.d.	n.d.	n.d.	n.d.
Content $CD19^+$ B-cells	FCM	< $1x10^4$ $CD19^+$ B cells/kg body weight of the patient	n.a.	n.a.	n.a.	n.a.
Sterility	Culture	Negative for bacterial and fungal contamination	negative	negative	negative	negative
Mycoplasm	Luminescence assay	Negative for mycoplasm contamination	negative	negative	negative	negative
Endotoxin	LAL assay	<0.25 EU/ml	0.08	0.02	0.01	0.01
Absence of cytokines	ELISA	< 25 pg/ml IL-2, IL-7, IL-15 and SCF.	yes	yes	yes	yes

P1059

Extracorporeal photopheresis as a second-line treatment of chronic graft-versus-host disease

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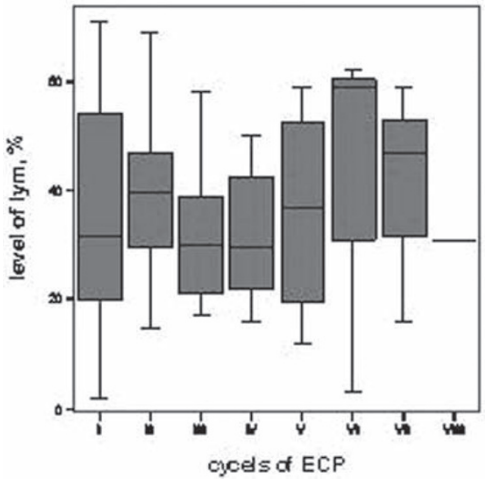
Chronic graft-versus-host disease (cGVHD) is the most serious and common long-term complication of HSCT, occurring in 20% to 70% of people surviving more than 100 days and it represents the major cause of nonrelapse mortality and morbidity in long-term survivors. At last years extracorporeal photopheresis (ECP) is used as a second line treatment in combination with other immunosuppressive drugs or alone in steroid-dependendt and refractory cGVHD.

Patients and methods: 18 pts (13 children and adolescents, 5 adults), age from 2 to 51 y.o. (median 22 y.o.) suffering from ALL – 5 pts, AML – 6 pts, biphenotyping AL – 1 pt, MDS – 1 pt, AA – 1 pt, CML – 3 pts, CLL – 1pt. All pts received allo-HSCT: matched unrelated HSCT – 7 pts (39%), mismatched unrelated HSCT – 3pts (17%), matched related HSCT – 5 pts (28%), haplo-HSCT – 3 pts (16%). Onset type of cGVHD is progressive (persistent sings and symptoms of aGVHD) – 3 pts (17%), quiescent (no clinical sings or symptoms of aGVHD) – 13 pts (72%), de novo (no prior history aGVHD) – 2 pts (11%). Corticosteroid status is dependent – 14 pts (68%), refractory – 2 pts (12%), intolerance (including severe myopathy, systemic viral, bacterial or fungal infections) – 2 pts (3%). Frequency of organ involvement: skin – 17 pts (94%), joints – 4 pts (22%), liver – 4 pts (22%), oral mucosa– 10 pts (55%), gastrointestinal – 6 pts (33%), eyes – 7 pts (38%), lung – 8 pts (44%), kidney – 1 pts (3%). ECP treatment: 2 times during 2 weeks or 2 times during 4 weeks. Patients received 1 to 10 cycles of ECP (median 4 cycles). After each procedure ECP level of lymphocytes and NK cells was analyzed.

Results: Complete resolution was not achieved in any pt. Partial resolution was achieved in 15 pts (83%). Steroid therapy could be discontinued in 3 of them (20%),discontinuation of steroid therapy with resolution of GVHD clinical sighs was observed in 2 pts (13%), decrease in steroid dosage with resolution of GVHD clinical sighs – 2 pts (13%), only resolution of GVHD clinical sighs – 8 pts (54%). Level of lymphocytes in peripheral blood count after all ECP cycles was the same. Therefore, ECP may be a method providing effective immunosupression without altering anti-infectious cell-mediated immunity. Level of NK cells has tendency to reduction.

Conclusions: ECP is effective method for treatment of steroid-refractory, steroid- dependent and steroid-intolerant cGVHD.



P1060

Chronic kidney disease after haematopoietic stem cell transplantation: a single-centre experience

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Objective: Hematopoietic stem cell transplantation (HSCT) is widely performed for treatment of malignancies and non-malignant hematologic disorders. Chronic kidney disease (CKD) is an uncommon but important complication, contributed by several risk factors. This study aims to determine the characteristic, etiology and outcome of patients with post-HSCT CKD.

Materials and methods: During the period November 1983 to December 2009, renal functions of post-HSCT adult patients with minimum follow-up of 100 days were studied. CKD was diagnosed according to the criteria of K/DOQI working group. The medical records of patients with post-HSCT CKD were reviewed retrospectively.

Results: In the 25-year period, nine of the 717 patients developed CKD after HSCT with a median of 29 months (range: 2-227 months), the incidence was 1.3%. Eight patients received allogeneic transplantation and one received autologous transplantation. For conditioning, three patients received non-myeloablative regimen, five patients had irradiation-contained regimen. Elevated creatinine level was found in seven patients and other two patients had nephrotic syndrome (NS) without decreased GFR. Of the five patients received renal biopsy, the results showed membranous nephropathy (N=2), hepatitis C virus related membranoproliferative glomerulonephritis (N=1), Chronic glomerulonephritis, graft-versus-host disease (GvHD)-related (N=1), and minimal change disease (N=1). Six patients had chronic GvHD at diagnosis, only one patient was maintaining immunosuppressant (IS) therapy. IS was re-initiated for four patients, resulting in improvement of two patients. One of the responder developed NS just after discontinuation of IS. Three patients progressed to advanced stage CKD finally, two of them received dialysis. The risk factors for post-HSCT CKD included chronic GvHD, nephrotoxic drugs use, radiation therapy, non-myeloablative transplantation, underlying comorbidity, and infection.

Conclusion: The development of post-HSCT CKD is contributed by multiple factors. Careful monitoring of renal function test is indicated. Renal biopsy remains essential to better understand the pathogenesis of CKD. Immunosuppressant may play a role in the management of post-HSCT CKD.

P1061

Stem cells therapy in radiation injuries from bench to bedside

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1. Radiotherapy may induce irreversible damage on healthy tissues surrounding the tumour. It has been reported that the majority of patients receiving radiation therapy show early or late tissue reactions of graded severity as radiotherapy affects not only the targeted tumor cells but also the surrounding healthy tissues. In Europe, per year, 1.5 million patients undergo external radiotherapy. Acute adverse effect concern 80% of patients. The late adverse effect of radiotherapy concern 5 to 10% of them, which could be life threatening. Eradication of these manifestations is crucial.
2. Effect of radiation on healthy tissue. The French Institute of Radioprotection and Nuclear Safety (IRSN) contribute to understand effect of radiation on healthy tissue. IRSN is strongly implicated in the field of regeneration of healthy tissue after radiotherapy or radiological accident and in the clinical use of cell therapy in the treatment of irradiated patients.
3. Initial success in cell therapy of bone-marrow micro-environnement. In collaboration with haematologist of Saint-Antoine Hospital (Paris, France), our first success in cell therapy was the correction of deficient haematopoiesis in two patients. The intravenous injection of Mesenchymal Stem Cells (MSC) has restored bone marrow micro-environnement after total body irradiation necessary to sustain haematopoiesis.
4. Foremost success in cell therapy of radiation induced burns. Cutaneous radiation reactions play an important role in radiation accidents, but also as a limitation in radiotherapy and radio-oncology. Recently, in collaboration with the Percy hospital (Clamart, France) we have evidenced for the first time, the efficiency of MSC therapy in the context of acute cutaneous and muscle damage following irradiation in five patients.
5. Promise approach for the medical management of gastrointestinal disorder after irradiation. We have demonstrated that MSC treatment is a promise approach for the medical management of gastrointestinal disorder after irradiation. We have shown that MSC migrate to damaged tissues and restore gut functions after radiation damage. Our group carefully studies side effects of stem cell injection for further application in patients.
6. Evaluation of stem cell therapy combining different source of adult stem cells is under investigation. Use of stem cells dedifferentiate from adult differentiated cells (IPS) will be soon tested in preclinical treatment of radio induced damage.

P1062

B- and T-cell interactions in graft-versus-host disease

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The role of B cells in the setting of GVHD remains controversial. We described a new interaction between autologous CD B cells and activated CD8 cytotoxic T lymphocytes (CTLs) in preliminary experiments on normal donors (Deola et a 2008). In vitro antigen-stimulated CTLs form stabilized couplets with B cells independently from antigen presentation. The effect of coupling enhances CTL survival and proliferation, and stimulates cytokine release by B cells, including Mig, IP-10 and I-TAC, which interact with the same receptor CXCR3, and are involved in inflammation and GVHD pathobiology. Studies on leukocyte migration and GVHD describe CXCR3 as pivotal for migration of T cells to GVHD target organs. Moreover Mig, IP10 and I-TAC play critical roles in cardiac allograft vasculopathy, and lung allograft rejection.

We analyzed B-T couplets in the blood of GVHD patients. Seventeen patients with active GVHD 3-105 months after BMT were confronted with 20 normal donors. B-T couplets measured by flow cytometry were present in both patients and normal samples, and when counted on lymphocyte number were significantly higher in GVHD patients (Table 1).

We also analyzed the presence of B and T cells in 6 biopsies of GVHD, obtained at 3-13 months after BMT (Table 2). Six biopsies without features of GVHD were also included. Biopsy specimens were double-immunostained for CD8 and CD20. Four out of 6 GVHD+ biopsies showed a limited number (up to 10) of B cells, at least focally coupled with CD8 T cells. Two further GVHD+ were negative for CD20. Four out of 6 GVHD- were CD20-. Two further GVHD- samples showed an evident CD20+ and CD8+ infiltration with an aspecific inflammatory pattern, and no evidence of couplets was present. Noteworthy, both patients were affected by inflammation involving the analyzed organ. None out of 3 control biopsies, taken from transplanted patients without signs of GVHD at 4-15 months after BMT resulted positive for B cells.

We suggest that the interaction between CTL and B cells could play a role in GVHD and inflammation target organs by amplifying the inflammatory signal, both in peripheral blood, and in the target organ. B cells contribution could act by locally attracting additional immune cells, and supporting CTL cells survival and expansion. Further experiments are undergoing to confirm these preliminary results on a larger sample size, and to distinguish the cytokine patterns released in GVHD and inflammation settings.

TABLE 1

Sample	# of Lymphocytes	# of CD8	# of CD19	% CD3/8/19	% CD3/8/19 on # of Lymphocytes x1000	% coupled CD8	% coupled CD19
GvHD (N=17)							
MEAN	1822,0	763,7	261,1	0,444	0,327	$8,38^{E-04}$	$1,77^{E-03}$
SEM	251,3	121,4	46,5	0,143	0,105	$3,59^{E-04}$	$4,33^{E-04}$
ND (N=20)							
MEAN	2222,6	717,6	229,5	0,257	0,128	$2,12^{E-04}$	$7,24^{E-04}$
SEM	169,2	74,0	28,9	0,035	0,021	$4,57^{E-05}$	$2,12^{E-04}$
p	0,17	0,73	0,54	0,159	**0,04**	0,055	**0,02**

LEGEND TO TABLE 1

GvHD = inclusion criteria: active GvHD, 3-105 months after BMT, at least 50 Cd19 cells/ul

= absolute number/ul

% coupled CD8= % CD8 coupled / CD8 absolute number

% coupled CD19= % CD19 coupled / CD19 absolute number

SEM = Standard Error Mean

p = 2 tails T-Test

TABLE 2

Disease	Source of BMT	Months after BMT	CD20+	Couplets	Histology (grading)	Involved organ
T-NHL	MUD PBSC	13,3	++	+	aGVHD (II)	SKIN
AML	MUD PBSC	2,8	+	+	aGVHD (II)	GI
AML	MUD BM	3,7	NEG	NEG	aGVHD (III)	SKIN
ALL	MRD BM	6,2	++	+	aGVHD (III)	SKIN
CLL	MRD PBSC	2,7	NEG	NEG	aGVHD (III)	SKIN
ALL	MRD BM	2,8	+	+	aGVHD (III)	SKIN
AML	MRD BM	6,9	+++	NEG	No GVHD (*)	GI
AML	MRD BM	16,8	NEG	NEG	No GVHD (*)	SKIN
MM	MRD PBSC	2,8	+++	NEG	No GVHD (*)	GI
AML	MRD PBSC	15,0	NEG	NEG	No GVHD (**)	NONE
AML	MRD PBSC	4,9	NEG	NEG	No GVHD (**)	NONE
AML	MRD PBSC	4,1	NEG	NEG	No GVHD (**)	NONE

LEGEND TO TABLE 2

NO 0 CELLS CD20/SLICE.

\+ >1 <=3 CELLS CD20/SLICE

++ >3 <=10 CELLS CD20/SLICE

+++ >10 CELLS CD20/SLICE REACTIVE PATTERN

(*) INFLAMMATION

(**) CONTROL BIOPSIES

P1063

High risk of PTLD development in lymphoma patients undergoing allogeneic haematopoietic stem cell transplantation

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Background: Allogeneic hematopoietic stem cell transplantation (SCT) is an important treatment modality in young patients with relapse of lymphoma. Post-transplant lymphoproliferative disease (PTLD) is a serious complication after solid organ transplantation and also after allogeneic SCT with high mortality especially if effective treatment starts late. Patients who undergo allogeneic SCT due to lymphoma may have especially high risk to develop PTLD. Previously, PTLD was often misdiagnosed as relapse, but strict criteria for the PTLD diagnosis have now been established. Our aim was to study the risk of PTLD development and find risk factors in patients transplanted due to lymphoma.

Patients and methods: All patients who underwent allogeneic SCT in all Swedish SCT centers due to lymphoma or chronic lymphocytic leukemia between 1984 and 2007 have been included. All patients who developed PTLD or relapse of lymphoma after the SCT were studied by reviewing patients' data records including pathology reports. Analysis of risk factors for PTLD or lymphoma relapse was performed.

Results: Fourteen out of 200 (7%) patients developed PTLD; 8 males and 6 females, median age 39 years. In total, 11 of these patients had received reduced intensity conditioning and 3 myeloablative conditioning. Only 2 had mismatched donors. 11 patients received ATG. Lymphoma patients with a longer duration between diagnosis and SCT showed a higher risk of development of PTLD (p=0.02). Patients with autologous SCT before allogeneic SCT also seemed to have a higher risk for PTLD, 8 patients (57%) versus 62 patients (33%) (p=0.08). All PTLD patients were EBV DNA positive at time of diagnosis (median 28 days) and 8 patients (57%) had a CMV infection. 11 of 14 PTLD patients had acute GVHD before development of PTLD; 7 grade I and 4 grade II-IV. In total, 3 of the PTLD patients had received rituximab (21%) as a part lymphoma treatment before SCT. The median of PTLD diagnosis 66 days after SCT (min 26, max 3375). All patients received riruximab as treatment of PTLD and 3 patients were also treated with chemotherapy. 9/14 (64%) died of PTLD. 68/200 (34%) developed relapse of lymphoma. The median time of relapse 233 days (min. 11, max. 2463), 68% died of relapse. There was a significant difference in cumulative proportion surviving between patients that developed PTLD and non-PTLD patients (p= 0.024).

Conclusion: Patients with lymphoma before allogeneic SCT were at risk of development of PTLD.

P1064

Disease status but not histology is predictive of outcome in lymphoma patients after allogeneic stem cell transplantation with reduced-intensity conditioning regimen

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Background: Lymphoma patients with disease relapsed after HDC or with disease refractory to several line of treatment can be rescued with allogeneic stem cell transplantation. The aim of this analysis was to report the results obtained in a cohort of lymphoma patients treated with reduce intensity conditioning regime (alloRIC) in a single institution.

Patients and methods: From 2001 and 2009, 113 lymphoma patients received alloRIC. Main patient characteristics are reported in the table Most patients received an association of fludarabine (30 mg/m²/day, 5 days), oral or intravenous busulphan (4 mg/kg or 3.2 mg/kg/day respectively, over 2 days), and thymoglobulin (2.5 mg/kg/day over 1 or 2 days). For these patients, graft versus host disease prophylaxis (GVHD) was cyclosporine (CyA) alone starting from days -3 at 3 mg/kg/day. Second most used conditioning consisted of fludarabine (30 mg/m²/day for 3 days) and low dose total body irradiation (TBI, 2 grays). For these patients, GVHD prophylaxis consisted of CyA and mycophenolate mofetil (MMF).

Results: After a median observation time of 28 months, the 3-year OS and PFS were 59% (CI 48%-68%) and 51% (CI 41%-61%), respectively. At last follow-up, 66 patients (58%) were alive and 47 patients (42%) died. The treatment related deaths were consequence of aGVHD 36% (11/30), cGVHD 23% (7/30), microbiologically not documented pneumonitis 10% (3/30), viral encephalitis 7% (2/30), graft failure 7% (2/30), SNC haemorrhage 1, cerebral aspergillosis 1, neoplasia 2 pts. One patient died of aGVHD after a second alloRIC for progressive disease. Univariate analysis showed that CR status at time of alloRIC was significantly associated to a better OS and PFS. Histological subtype did not influence the OS.

aGVHD incidence was 43%, grade II-IV was 38% (65% grade 2, 18% grade 3, and 17% grade 4), and the median time of diagnosis was 33 days (CI 11-114).

	N=113
Median age (range)	48 (17-68)
F/M	
Histology	
HL	22 (19%)
NHL	91 (80%)
Aggressive	35 (32%)
Mantle	16 (18%)
Low grade	40 (40%)
Previous HDC	
Yes	81 (72%)
No	32 (28%)
Disease status at alloRIC	
CR	67 (60%)
PR	33 (29%)
SD/PD	13 (11%)
Donor type	
HLA identical sibling	97 (86%)
MUD 10/10	9 (8%)
MUD 9/10	1 (1%)
CBU	6 (5%)
Sorror score*	
0	34 (32%)
1-2	40 (38%)
≥ 3	32 (30%)
Conditioning regimens	
FBS	75 (66%)
FTBI	24 (21%)
Others	14 (13%)
ATG	
Yes	75 (66%)
1 day	45 (60%)
2 days	25 (33%)
≥ 4 days	6 (7%)
No	38 (34%)

The cGVHD incidence was 33% (35/106), and the incidence of extensive form was 71%. Cytomegalovirus infection was detected in 13 patients (11%) at the median time of 34 days (CI 1-83) after alloRIC. Only one gastro-intestinal CMV disease was observed. The 100-day and 2-year TRM was 6% and 28% (CI 20%-35%), respectively.
Conclusion: This retrospective analysis showed that, contrary to other studies, histological subtype did not present a major impact on outcome. Clinical results appear promising with a low TRM and rather high outcome in a cohort of poor prognosis lymphoma patients.

P1065
Reduced-intensity conditioning regimen with ex vivo T-cell depletion after unrelated donor as stem cell source for patients with haematological malignancies
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Background: Most patients received allogeneic stem cell transplantation from matched unrelated donors after myeloablative or reduced intensity conditioning regimen (alloRIC). Using T cell replete peripheral blood stem cell (PBSC), graft versus host disease (GVHD) was of concern. Extensive T cell depletion could blunt the graft versus malignancies effect. The aim of this retrospective analysis was to depict the outcome of patients after iv busulfan and ATG-based RIC and unrelated donor.
Patients and methods: Since 1998, we performed 537 alloRIC and 116 (22%) were conditioned by fludarabine, iv busulfan and anti-thymocytes globulin (ATG) (FBA). Of these, for 42 patients (34%) donor was unrelated. Conditioning regimen consisted of fludarabine (150 mg/m²), iv busulfan (6.4 mg/kg), and intermediate dose of ATG (5 mg/kg), day -2 and -1. PBSC were used in all but 4 patients. GVHD prophylaxis consisted of cyclosporine alone starting day -3. Cytomegalovirus was managed as pre-emptive strategy, based on weekly antigenemia. No anti-molds active prophylaxis was performed. Main patient characteristics were in Table.

	N 42
Median Age	54 (27-68)
Sex M/F	27/13
Disease	
AML/MDS	21 (50%)
ALL	2
IMF	2
MM	7
Lymphoma	6
CLL	4
Disease status	
CR/PR	30 (71%)
Active disease	12 (29%)
Donor	
10/10	33 (79%)
9/10	9 (21%)
PBSC	38
BM	4
Sorror score*	
0-1	12 (31%)
2	11 (28%)
>3	16 (41%)

Results: The median observation time was 27 months (range 5-50). One patient experienced graft failure and died. The median time to ANC more than 0.5 x $10^9$and platelets more than 20 x 10^9 was 18 days and 12 days, respectively.
Acute GVHD grade 2-4 and grade 3-4 was 42% and 16%, respectively. Chronic GVHD incidence (in 37 evaluable patients) was 51% (extensive 68%).
During aplasia period, 71% of patients had fever of unknown origin, 6% sepsis, and 18% did not developed fever. CMV reactivation was observed in 55% of patients and 2 patients developed CMV colitis; 31% had multiples reactivation. EBV reactivation, with no lymphoproliferative.

P1066
Haematopoietic stem transplantation for primary immunodeficiency disease: a south-east Asian perspective
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Haematopoietic stem cell transplants (HSCT) is the only cure for primary immunodeficiency disease (PID). If left untreated, PID is associated with high mortality from serious infections in childhood.Since the 1990's cord blood forms an important source of stem cells for our 2 paediatric transplant programmes. We described our experiences on HSCT for children with PID in our population.
Method: We performed HSCT for 14 children with PID over a period of 12 years from Dec 1996 to Feb 2009. Of these,10/14 (71%) received unrelated cord blood transplants (CBT), 3/14 had MSD and only one had MUD bone marrow transplant. The underlying diseases included SCID(5), HIgM(5), CGD(2),WAS(1), LAD(1).The median age at transplant was 35 months (range 3 to 204 months). Conditioning regimes consisted of myeloablative Busulphan (BU)/Cyclophosphamide (CPA)±ATG regimes for all except for the 5 SCID who had reduced intensity conditioning (RIC). The RIC regime consisted of Fludarabine ± Melphalan ±CPA.GVHD prophylaxis consisted of cyclosporine A and/or a combination of short course of methotrexate, methylprednisolone or mycophenolate mofetil.
Results: Fourteen percent (2/14) had graft rejection:one pt with HIgM had a 2nd successful transplant from the same MUD and one pt with GCD died 1.5 year later from pneumonia secondary to his underlying disease after refusing 2nd transplant. TRM was 14% (2/14) both had unrelated CBT, one died from pneumonia and the other from chronic GVHD in liver and lung. Overall survival remained good at 79%(11/14).Of those with unrelated CBT ,7/10 (70%) are alive. Pre and post transplant infective complications were significant in our patients, it ranged from disseminated BCGitis seen in SCID cases to systemic fungal infections.
Conclusions: PID is highly curable with HSCT.MSD transplants give 80 to 90% while MUD gives 50 to 60% cure reported in literature.The unrelated CBT in our study showed a intermediate range of cure 70% .In a diverse racial groups such as ours, it is often difficult to find a bone marrow match in international registries. Unrelated cord blood transplant gives these children with PID a good chance for cure.

P1067
Haemorrhagic cystitis in paediatric haematopoetic stem cell transplant: report from a paediatric transplant centre in Singapore
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Haemorrhagic cystitis is a known morbidity in the haematopoietic stem cell transplant (HSCT) setting. We describe the cases in our institute in the last 12 years.

Methods: This is a retrospective chart review of HSCT patients in our institute from 1998 to 2010 (12 years). Data on patient characteristics, type of HSCT as well as description of the hemorrhagic cystitis episodes, their management and outcomes were collected.
Results: There were 8 patients with haemorrhagic cystitis in the 12 years. During this period 97 cases of HSCT were performed in our center. This give a incidence of 8 % in our study. The age ranged from 3 to 13 years. There were 4 girls and 4 boys. Majority (7/8, 88%) had allogeneic HSCT. The indications for the HSCT in these patients were: chronic myeloid leukemia (2 patients), myelodysplastic syndrome (2 patients), acute lymphoblastic leukaemia (2 patients), acute myeloid leukaemia (1 patient) and high-risk neuroblastoma (1 patient, autologous HSCT). Conditioning for all the allogeneic HSCT included cyclophosphamide – BuCy (4 patients) or CyTBI (3 patients). The onset of hemorrhagic cystitis ranged from day +5 to day +68. Duration was 3 to 153 days. Urine BK virus was positive in 5 patients (urine BK virus studies were not performed for first 3 cases). Two of these received intravenous (IV) with/without intravesical cidofovir, with unsatisfactory results. All patients received blood product support for thrombocytopenia. Five (62 %) patients, 2 of whom had BK viruria, required urine catherization and bladder washout and eventually underwent cystoscopy and diathermy. One of these 5 patients needed a suprapubic catheter. Another one underwent intravesical prostaglandin (PGF2 α) with disappointing results. Two patients, both BK positive, had spontaneous resolution.
Conclusion: From our series, haemorrhagic cystitis was a significant morbidity in HSCT patients. Most of these patients had to undergo invasive interventions such as cystoscopy and diathermy, and intravesical drugs. BK viruria is a common cause of haemorrhagic cystitis in our study. Optimal management of this problem is still unknown.

P1068

Immunotherapy with cytokine-induced killer cells in patients with refractory solid tumours

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This study aimed to evaluate the feasibility to expand Citokine Induced Killer (CIK) cells from heavily pre-treated neoplastic patients and to assess the toxicity of this therapeutic treatment. The investigation was approved by the Ethical Committee and the informed consent was provided by the patients enrolled in the study. Peripheral blood mononuclear cells (PBMC) were obtained by apheresis in 4 patients with advanced refractory cervical cancer and 1 patient with methastatic melanoma. PBMC were cultured in bags (Mylteny/Cell Genix) by using serum free X-vivo medium (Biowhittaker) initially additioned with IFN-γ (Imukin Boehringer Inghelheim) 1000UI/mL and 24 hours later with Thymoglobulin (Genzyme) 500 ng/mL and IL-2 (Proleukin, Novartis) 300 UI/mL. The expansion of CIK was then sustained by only adding IL-2. Phenotypic characterization was performed weekly using FAC scalibur and the following moAbs: CD3,CD4,CD8,CD56,CD25, foxP3 (Becton Dickinson). The sterility tests were done using BactAlert System and both aerobic and lytic anaerobic media (Biomerieux). Endotoxin was evaluated by Limulus Amebocyte lisate, LAL test (Cape Code) with a sensitivity of 0.06 UE/mL. Cell viability was evaluated at the end of the culture before criopreservation and checked before thawing and infusion.
At least 3 doses were obtained for each patient and the number of CIK ranged from 1 to 6 x 10^6 cells/kg based on the fold expansion. One patient died during the expansion procedure for complications due to primary tumour. The other patients received a minimum of 3 cycles of infusions with an interval of 1 month between the infusions. The culture performed under GMP grade was successful and the quality controls indicated that the product was sterile throughout the entire culture period. Cell viability at the end of expansion and following cryopreservation ranged between 80-98%. The protocol treatment was safe without any toxicity following each cell cycle administration. After a follow-up of 8-12 months, 2 patients are still alive with a slow progressive disease whereas 2 patients died 4 months later the immunotherapy treatment.
Even though the patients enrolled in this preliminar study had very critical clinical conditions, it was possible to expand an appropriate number of CIK cells. Within the limit of the study, this therapeutic approach seems to be feasible and no toxic, it could be a promising treatment in patients with more limited tumour burden.

P1069

Enhanced secretion of high mobility group Box 1 (HMGB1) by haematopoietic stem cells after nucleofection and femtosecond laser-transfection

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Background: Genetic modifications of hematopoietic stem cells (HSC) contain a high potential to gain insights into the pathogenesis and new therapeutic approaches of various diseases. High mobility group box1 (HMGB1) is a proinflammatory cytokine, which stimulates immune reactions. Here, we investigated whether canine HSC (CD34+) can be transiently transfected with a 5.3 kb plasmid containing genetic information for HMGB1 by two different methods: nucleofection and femtosecond (fs) – lasertransfection. The latter one is based on short laser pulses triggered on cell membranes to produce micropores allowing the uptake of external plasmids.
Methods: HSC were enriched from canine bone marrow by magnetic activated cell sorting. Thereafter, two transfection approaches were investigated: A) nucleofection (AMAXA® device) and B) fs-lasertransfection (0.1-0.3 J/cm^2). As plasmid an eukaryotic expression vector pEGFP-HMGB1 containing a CMV promotor was applied. Transfection rates were detected by flow cytometry and fluorescence microscopy. Levels of secreted HMGB1 were analysed by ELISA. Subsequently transfected HSC were differentiated into dendritic cells (DC) (IMDM, supplements: TNFa [10 ng/ml], GM-CSF [40 ng/ml], FLT-3 [200 ng/ml]; harvest d11 to d15).
Results: The transfection rates were 12-25 % (nucleofection, n=3) and 1-5 % (fs-lasertransfection, n=3) after 24h, respectively. After nucleofection (24h) cell numbers decreased by 90-94 % (cell loss), 11-18 % of the cells were apoptotic and 43-80 % necrotic. In comparison after fs-lasertransfection (24h) cell numbers decreased by 56-79 %, 21-36 % of the cells were apoptotic and 12-35 % necrotic. HMGB1 release into the supernatant was highest early after transfection. After nucleofection HMGB1 levels were 6.6 ng/ml (d2), 3.4 ng/ml (d5) and 1.0 ng/ml (d9). In comparison HMGB1-supernatant levels after fs-lasertransfection were 23.4 ng/ml (d1), 12.3 ng/ml (d2), 0.9 ng/ml (d5), 1.0 ng/ml (d8) and 0.9 ng/ml (d14). Subsequent generation of HMGB1-expressing DC was possible after both transfection methods, but hampered by the high cell loss.
Conclusions: Canine HSC can be transfected with a HMGB1 plasmid by nucleofection as well as the novel fs-lasertransfection techniques, however cell loss is significant, so further improvement is warranted. HMGB1-producing DC can be differentiated from transfected HSC, which might be used in the future for cellular therapies in the setting of vaccination studies.

P1070

Pre-transplant serum cytokine profiles in allotransplanted patients

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Background: Several cytokines are altered in patients with hematologic malignancies, and such alterations may have direct effects on the malignant cells or have indirect effects on leukemogenesis through altered function of bone marrow stromal elements. However, the cytokine system constitutes an interacting functional network where the contribution from single cytokines is modulated by the levels of other cytokines. It may therefore be more relevant to look at the total serum cytokine profile.
Patients and methods: We investigated the pretransplant serum levels of 34 cytokines in a group of 40 consecutive allotransplanted patients (26 men, 14 women; median age 43 years (18-62 years)). The large majority of patients had acute leukemia (AML 25 patients, ALL 13 patients). Serum samples were collected before start of conditioning therapy when all patients were in complete hematological remission. All patients received myeloablative conditioning therapy and graft-versus-host disease (GvHD) prophylaxis with cyclosporine A plus methotrexate, and they were transplanted with G-CSF mobilized peripheral blood stem cells derived from HLA-matched family members.
Results: The serum cytokine profile showed considerable variation between the patients, and when using unsupervised hierarchical clustering analysis the patients could be divided into two major subsets. These two subsets differed especially in serum hepatocyte growth factor (HGF) levels, but Epidermal growth factor (EGF) and leptin levels also varied. The two subsets did not differ in age, diagnosis, diagnosis or frequency of patients with acute GvHD. The degree of weight gain/fluid retention after conditioning therapy did not differ between the two patient subsets. The pretransplant serum cytokine profile for the whole patient group differed from healthy controls, especially (i) increased G-CSF levels; (ii) decreased levels of several T cell-derived cytokines (e.g. IL2, IFN-γ); and (iii) altered chemokine levels. Thus, the cytokine profiles for the two patient subsets identified in the hierarchical clustering differed from each other and both differed from the healthy controls.
Conclusion: We conclude that the pretransplant serum cytokine profile shows a considerable variation even between patients in complete hematological remission, and patients can be divided into two major subsets based on this profile.

P1071

Haematopoietic recovery in old and young dogs

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Autologous stem cell transplantation is limited in elderly patients due to various comorbidities influenced by life style and prior treatment. We were interested to study hematopoietic recovery and clinical course independent of these factors in old dogs (between 8 and 12 years of age) as compared to young dogs (between 1 and 2 years of age). The animals received a single dose of 10 Gy total body irradiation and 1 x 10^8 mononuclear cells/kg of bone marrow previously aspirated from humerus and femurs and cryopreserved. In older dogs a significantly higher volume had to be obtained in order to reach the amount of cells. There was no difference in the rapidity of hematopoietic recovery and platelet counts did not differ between old and young dogs. Granulocyte and lymphocyte counts were lower prior to transplantation in old dogs as compared to young dogs and pretransplant levels of granulocyte counts were reached in both groups at day 40. Lymphocyte counts remained below pretransplant levels in both groups and abnormal in old dogs. The clinical course was complicated by diarhhea and fever in young dogs on days 5 and 6, in old dogs on days 4 -8 and later on days 11-14. These differences reached significance at the level of p=0.05.
The animal experiments indicate that a given amount of mononuclear cells containing similar cfu-c numbers leads to a similarly rapid recovery in old as in young dogs. Lymphocyte counts may remain low in both ages. In contrast gastrointestinal toxicity is more severe and of longer duration in old dogs. Elderly individuals may require more intensive gut decontamination and preventive measures of infections.

P1072

Posterior reversible encephalopathy syndrome

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Background: posterior reversible encephalopathy syndrome (PRES) is one of the serious adverse side effects of Cyclosporine A,Tacrolimus and Sirolimus, which are used for the prophylaxis of acute graft-versus-host disease (GVHD) after allogeneic stem cell transplantation (allo-SCT). We present 2 patients who developed PRES, after allo-SCT. The patients were female (24 and 28 years old) with AML, transplanted from sibling male donor. Ciclosporin A and MTX (1, 3, 6, 11 day) was used as aGVHD prophylaxis. Median onset of PRES was 22 and 24 days after allo-SCT.Primary symptom was headache and visual disturbance and subsequently development of systemic seizure. Serum creatinin was 166 mmol/L. and 180 mmol/L. Sites of PRES by MRI were detected in the temporal, parietal, frontal, and parietal lobes, and brain stem. Development of status epilepticus need ICU care. Now, 3 years after transplantation they are good with signes of chronic limited GVHD. These data suggested the importance of early intervention for PRES and exploitation of optimal GVHD prophylaxis after developing PRES.

Stem cell mobilization and Graft engineering

P1073

The role of the CXCR4 antagonist Plerixafor® in elderly patients failing autologous stem cell mobilization. Data from the Plerixafor Compassionate Use Study. On behalf of the European Consortium for Stem Cell Mobilization (ECOSM)

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Autologous stem cell transplantation (ASCT) is the single largest transplant procedure performed in Europe accounting for 60% of first transplants (EBMT 2008). Patients >60 years (yrs) of age constitute a significant and increasing proportion of all ASCTs. With increasing life expectancy combined with improved supportive care it is conceivable that the numbers of patients of advanced age suitable for a first ASCT for haematological malignancy will further increase. Increasing age has been shown to adversley affect CD34+ stem cells (SC) mobilization and collection and thus may impact on treatment options and overall outcome. Data suggest that the CXCR4 antagonist Plerixafor is useful in achieving adequate SC harvests in patients failing prior mobilization. Specific data on the use of this drug in elderly pts is lacking. We analyzed the impact of

age on SC collection in patients >60 yrs receiving Plerixafor as part of a European compassionate use programme following primary mobilization failure. A total of 498 pts with either myeloma (MM) or Non-Hodgkins lymphoma (NHL) underwent attempt at SC mobilization and collection, 244 (49%) were >60 yrs of age; 160 were between 60-65 yrs (B) and 84 >65yrs (C) (median ages 62 and 67 yrs respectively). The median age of patients receiving Plerixafor <60 yrs (A) was 53 yrs (16-59). There were no significant differences between the groups with respect to gender, number of lines of prior chemotherapy, use of prior radiotherapy or number of patients who subsequently underwent ASCT. We found, following Plerixafor salvage, no difference in the CD34 yield between patients <60 and those either 60-65 or >65 yrs (P=0.26 and P=0.31), median CD34+ stem cell yields were 3.1 (0.18-32.6), 3.4 (0-16), and 2.7x10^6/kg (0.1-10) for groups A, B and C respectively. 61%(A), 68% B),and 66% (C)respectively achieved the primary end point of the study i.e to collect ≥2.0x10^6/kg CD34+ SC allowing a single ASCT procedure. As MM was increased in patients >60,53% and 57% in groups B and C compared to those <60(38%),we investigated whether this masked an inferior yield in older patients with NHL. No difference was seen between patients with NHL in group A vs groups B and C (P=0.39 and P=0.168 respectively). In conclusion we demonstrate that in almost 250 patients >60 years failing SC mobilization Plerixafor treatment allows collection of adequate CD34+ (2.0x10^6/kg) cells to allow a ASCT. Patients >60 yrs should therefore be considered for treatment

P1074

Successful eradication of acute myeloid leukaemia blasts by single-locus HLA-mismatched leukaemia-reactive CD8+ T-lymphocytes in a humanized NOD/SCID/IL2Rgcnull xenograft model

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Donor lymphocyte graft engineering to avoid graft-versus-host (GVH) reactivity while improving graft-versus-leukemia (GVL) immunity remains of central interest in allogeneic hematopoietic stem cell transplantation. To evaluate new experimental concepts of donor lymphocyte allograft engineering to improve GVL reactivity of human T cell-grafts to acute myeloid leukemia (AML) in vivo, we established a humanized transplantation model using immunodeficient NOD/SCID/IL2Rgcnull (NSG) mice and AML samples from patients. Here, we report the successful eradication of engrafted AML blasts from patient MZ580 using donor-derived, single HLA-B locus-mismatched, AML-reactive CD8+ T cells generated in mixed lymphocyte leukemia cultures (MLLC). Upon injection of 5x10^5 primary blasts into 6 to 8 week old irradiated (150cGy) NSG mice, 1% AML engraftment in the bone marrow (BM) was achieved within 18 days, resembling minimal residual disease. 5x10^6 AML-reactive CD8+ T cells derived from MLLC cultured for 14, 21, 28, and up to 56 days were adoptively transferred 18 days post AML engraftment to investigate differences in homing, survival and GVL reactivity in vivo. Additionally, cytotoxicity to AML blasts was analyzed in a kinetic experiment in leukemia bearing mice 2h, 24h, 48h and 7 days post T cell transfer. To study antileukemic responses of administered T cells ex vivo, reisolated splenocytes from T cell-treated mice were tested in vitro by IFNγ(g)-EliSPOT assays. We observed complete eradication of AML blasts in BM, spleen and peripheral blood of tumor bearing mice one week after transfer of CD8+ T cell lines cultured for 14, 21, and 28 days, whereas controls showed 25-61% (median 35%) AML engraftment in the BM. Kinetic analysis demonstrated nearly complete eradication of AML blasts as early as 48h after T cell transfer. Ex vivo analyses of T cells recovered from spleens 24h and 48h after injection showed highly specific reactivity against AML MZ580 blasts in IFNg-EliSPOT assay, whereas NSG DCs were not recognized. However, T cells cultured for 56 days appeared less capable to eradicate AML blasts in vivo. Further experiments to optimize conditions required for effective homing, survival and central memory of leukemia-reactive T cell-grafts also in a HLA-matched setting are currently in progress.

In summary, these results suggest that our model might represent a valuable tool to define conditions for optimized GVL immunity of leukemia-reactive T cell-grafts in vivo.

P1075

Plerixafor for stem cell mobilization in Hodgkin, non-Hodgkin, and multiple myeloma patients: a subgroup analysis from the European Compassionate Use Program

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The CXCR4-inhibitor plerixafor mobilizes hematopoietic stem cells (HSC), thereby amplifying the effects of G-CSF. Before approval plerixafor was evaluated in compassionate use programs (CUP) in patients who failed a prior mobilization. Thirteen European countries enrolled a total of 510 patients (226 Non-Hodgkin lymphoma (NHL), 235 Multiple Myeloma (MM), 49 Hodgkin lymphoma (HL)) who received 0.24 mg/kg bodyweight (BW) plerixafor subcutaneously (SC) 9-11 hours prior to apheresis.

Two hundred twenty-six NHL patients (112 male, 114 female; median age 62 years, range 16-75; median lines of pretreatment: 2; range 0–10) collected a median of 2.51 x 10^6 CD34+ cells/kg BW (range 0–15.99). Onehundred-fifty patients (66.4%) gathered the defined minimum of 2.0 x 10^6 CD34+ cells/kg BW in a median of 2 apheresis sessions (range 1–5). Sixty-six patients (29.2%) yielded more than the minimum amount of CD34+ cells during the first apheresis session. 64.6% of patients proceeded to high-dose chemotherapy followed by autologous stem cell transplantation and received a timely and stable engraftment.

The group of 235 MM patients (135 male, 100 female; median age 61 years, range 28 – 76; median lines of pretreatment: 2, range 0–9) collected a median of 3.59 x 10^6 CD34+ cells/kg BW (range 0.1–32.6). A total of 194 patients (85.8%) yielded enough CD34+ cells/kg BW to proceed to transplantation. Ninety-four patients (40%) gathered the minimum amount of CD34+ cells during the first apheresis session.

Twenty-four male and 25 female with a median age of 39 years (range 19–75) received a median of three prior lines of therapy (range 2–5). Thirteen patients had received radiation treatment. In a median of 2 apheresis sessions (range 1–4) a median of 3.12 x 10^6 CD34+ cells/kg BW (range 0.1–8.62) was yielded. 85.7% (42/49) of HL patients gathered the minimum amount of CD34+ cells to proceed to high-dose chemotherapy followed by autologous stem cell transplantation and received a timely and stable graft. Nineteen patients (38.8%) gathered more than the minimum during first apheresis session. In total 386 patients (75.7%) of the evaluated 510 proven poor mobilizers gathered the defined minimum of CD34+ cells. The minimum stem cell collection targets were reached for over 80% of MM and HL patients and 2/3 of NHL patients. Observed adverse events were rare, mild and manageable. Our data might be of interest for the development of future mobilization strategies.

P1076

Ex vivo expansion of megakaryocytic progenitor cells to alleviate thrombocytopenia following multiple cycles of high-dose chemotherapy for highly malignant sarcoma

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Aggressive combination chemotherapy has dramatically improved the prognosis of osteosarcoma and Ewing's sarcoma. Dose-response relationships for individual chemotherapeutic agents have been demonstrated, and maintaining high dose intensity throughout the treatment is of proven importance for outcome. However, a recent analysis of toxicity demonstrated that about 25% of chemotherapy courses were delayed because of protracted thrombocytopenia. The present protocol was developed to explore whether ex vivo expanded megakaryocytic progenitor cells can substantially alleviate thrombocytopenia following multiple cycles of non-ablative high-dose chemotherapy for sarcoma, making it possible to maintain optimal dose intensity throughout the treatment. Moreover, HSC are normally maintained and proliferate under low O2 tension in BM and by mimicking these in vivo conditions in a special minilaboratory for hypoxia we want to explore if ex vivo expansion of HSCs under these conditions is more efficient than our previously normoxia ex vivo conditions.

Peripheral blood progenitor cells were harvested and a CD34+ selection performed (98% purity) after two courses of chemotherapy. CD34+ cells were expanded for seven days in serum-free media supplemented with thrombopoietin, kit ligand, flt3 ligand, IL-3 and IL-6, and reinfused if a prior episode of grade IV thrombocytopenia had occurred. Seven ex vivo expansions and reinfusions have been performed on three patients. No side effects were associated with the reinfusion of expanded cell products. The results presented represent the mean ± SEM of pooled data from seven ex vivo expansions. Total nucleated cells were expanded 9.4 ± 2-fold, whereas CD34+ progenitors and CD61+ megakaryocytic cells were expanded 5.7 ± 2-fold and 71,8 ± 31-fold, respectively. The progenitor cells CFU-Mk, CFU-GM, and BFU-E were expanded 3.5 ± 1-fold, 6.2 ± 1-fold and 2.7 ± 0.4-fold respectively. Of interest, the number of long-term culture-initiating cells did not increase, but were maintained at initial levels. We are currently exploring whether culturing the CD34 positive cells under hypoxic conditions would be more efficient for the expansion of the HSC, and data will be presented.

So far, we have demonstrated the feasibility of this clinical-scale ex vivo expansion protocol. Reinfusion of the expanded cell products has resulted in rapid recovery of platelet function, and no severe side effects were observed.

P1077

Autologous haematopoietic stem cell mobilization with plerixafor and G-CSF: impact of overweight and obesity

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Autologous hematopoietic stem cell mobilization was suggested to be influenced by overweight and obesity. Moreover, some recent data suggested that hypercholesterolemia can be associated with improved CD34+ yield (Crysandt et al. ASH2010). In order to assess the impact of obesity as a predictive factor in the context of autologous stem cell mobilization using the CXCR4 antagonist, plerixafor, we performed a large analysis in a group of 359 adult patients mobilized with plerixafor plus GCSF following previous failed mobilisation in whom data were available regarding BMI and CD34+ cell yield. 48% of these patients were diagnosed with multiple myeloma, 42% with non-Hodgkin's and 8% with Hodgkin's disease. Median age was 59 years, and 53% were males. The median number of prior chemotherapy regimens was 2 (range, 0-9), with the latter incorporating melphalan in 17%, lenalidomide in 5% and purine analogs in 8% of cases. The median baseline platelet count was $153x10^9$/l (range, 34-397). The median body weight was 74 (range, 43-132) Kg and the median body mass index (BMI) was 26 (range, 15-47). We analyzed CD34+ stem cell mobilization and harvest outcome in 2 cohorts of patients based on BMI: <25 (normal and underweight, N=134) and ≥25 (overweight and obese, N=225). Characteristics of patients from both groups were comparable, except for gender (more males in the BMI≥25 group). After first injection of plerixafor, the peripheral blood CD34+ cell count was significantly lower in patients with BMI≥25 vs those with BMI<25 (25±24 vs 55±64 cells/ul; p=0.04). Among patients who proceeded to aphereses, the single apheresis yield was also significantly lower in the BMI≥25 group ($2.0±1.6x10^6$ CD34+ cells/kg) than in the BMI<25 group ($2.6±2.6x10^6$ CD34+ cells/kg; p<0.05). After a median of 2 aphereses (range, 1-5) patients with BMI≥25 collected an inferior total yield of CD34+ cells ($3.5±2.0x10^6$ cells/kg) than patients with BMI<25 ($4.3±4.2x10^6$ cells/kg, p=0.053). However, comparable proportions of patients from both groups achieved the target of $2.0x10^6$ CD34+ cells/kg (76% of patients with BMI≥25 vs 78% with BMI<25). We conclude that autologous stem cell mobilization with plerixafor is significantly impacted in overweight and obese patients. However, it is likely that plerixafor can overcome this limitation, since this did not influence the ability to collect sufficient numbers of CD34+ stem cells allowing to perform at least one transplant procedure.

P1078

Plerixafor might overcome the detrimental effect of previous autologous stem cell transplantation on haematopoietic stem cell mobilization in myeloma patients

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A proportion of patients with multiple myeloma (MM) who have already undergone autologous stem cell transplantation (autoSCT) have indications for subsequent autoSCT. If they do not have a stem cell product available, they need to undergo another round of stem cell mobilization. Plerixafor is a CXCR4 receptor inhibitor, and is used in combination with granulocyte-colony stimulating factor (G-CSF) for stem cell mobilization in patients who show poor mobilization. We analyzed retrospectively the outcomes of stem cell mobilization with plerixafor and G-CSF in a group of 68 patients with MM: 25 patients who had undergone autoSCT previously [autoSCT(+)], and 43 other patients with MM [autoSCT(-]. The autoSCT(+) patients differed from the autoSCT(-) patients in several respects that could affect stem cell mobilization negatively: they had undergone an extensive number of chemotherapy courses (median 17 vs 6.5) and regimens (median 6 vs 2) and more frequent treatment with lenalidomide (27% vs 5%), and had a lower baseline leukocyte (median 3.8 vs 4.6 G/L) and platelet count (median 121 vs 194 G/L). We observed that the mean peripheral blood (PB) concentration of CD34+ cells after the first administration of plerixafor was lower in the autoSCT(+) patients (mean 21±13 cells/uL) than in the autoSCT(–) patients (mean 38±33 cells/uL, p=0.051). The mean yield of the first apheresis was slightly lower in autoSCT(+) patients (mean $2.1±2.1x10^6$ CD34+ cells/kg) than in autoSCT(–) patients (mean $2.8±2.7x10^6$ CD34+ cells/kg). As a consequence, the minimum target of $2.0x10^6$ CD34+ cells/kg was collected from 28% of autoSCT(+) patients on the first day of apheresis, compared with 53% of the autoSCT(–) patients (N.S.). However, this was improved on subsequent days and successful cell collection was achieved in a similar proportion of autoSCT(+) and autoSCT(–) patients (76% vs 81%, respectively, N.S.). In those patients who underwent

autoSCT after plerixafor mobilization, the median time to neutrophil recovery (≥0.5 G/L) was 12 days (range, 9–41) and to platelet recovery (≥20 G/L) 13 days (range, 10–41), which did not differ between the groups. Our observations suggest that stem cell mobilization with plerixafor and G-CSF might overcome the negative impact of prognostic factors for poor stem cell mobilization in patients with MM who have undergone autoSCT previously.

P1079

Plerixafor boost in association with chemotherapy based mobilization. Comparison with mobilization without chemotherapy

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Plerixafor is a selectable CXCR4 receptor antagonist which is used in combination with G-CSF to rescue hematopoietic stem cell mobilization in poorly mobilizing patients. However, the plerixafor booster could also be used to rescue the outcome of chemotherapy-based mobilization regimens. Therefore, we retrospectively analyzed this problem in a group of 197 patients. Fourty eight patients (24%) have been treated with plerixafor in combination with chemotherapy and G-CSF [chemo(+)], while 149 patients (76%) have received the more traditional regimen of plerixafor and G-CSF [chemo(-)]. In the chemo(+) patients, the first dose of plerixafor was administered after median of 13 days (range, 9-47) after chemotherapy and 11 days (range, 5-44) of G-CSF treatment. On the day of first plerixafor injection, the mean PB CD34+ cell count was 8±8 cells/uL in chemo(+) patients, and tended to be higher in chemo(-) patients (13±13 cells/uL, p=0.07). However, the day 1 PB CD34+ cell concentration did not differ between groups and was 31±43 CD34+ cells/uL in chemo(+) arm and 33±34 CD34+ cells/uL in chemo(-) arm (N.S.). The patients underwent median of 2 aphereses, ranging from 1 to 4, which was significantly less in chemo(+) than in chemo(-) patients ($p<0.05$). In turn, the mean single apheresis yield was significantly higher in chemo(+) (3.2±2.8 x 10^6 CD34+ cells/kg) than in chemo(-) patients (2.1±2.1 x 10^6 CD34+ cells/kg, $p<0.05$). Finally, both groups collected comparable number of CD34+ cells [4.5±2.6 x 10^6 CD34+ cells/kg in chemo(+) vs 4.5 ± 4.5 x 10^6 CD34+ cells/kg in chemo(-) patients, N.S.]. The proportion of patients, who collected the minimum target of 2.0 x 10^6 CD34+ cells/kg was also similar in chemo(+) (70.8%) and chemo(-) patients (66.4%, N.S.). However, we observed that plerixafor was most efficient when used to rescue the first line chemotherapy-based mobilizations, and then even 90.9% of patients collected 2.0 x 10^6 CD34+ cells/kg in this setting, compared with 54.2% of chemo(+) patients who received plerixafor boost after previous mobilization failure ($p<0.01$). We did not find any correlation between the leukocytosis on the day of first plerixafor injection or number of days on G-CSF treatment and mobilization efficacy. Our analysis has shown that plerixafor boost for the rescue of chemotherapy-based mobilizations is associated with higher yield and lower number of aphereses required to achieve the target.

P1080

A proactive approach to plerixafor use and subsequent apheresis can reduce failed autologous PBSC mobilization rates to virtually zero after two mobilization attempts: a single-centre series of 40 consecutive PBSC mobilization episodes using plerixafor

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Approximately 15 to 20% of patients ideally requiring autologous PBSC transplant fail to mobilize adequate PBSC doses by conventional means. Plerixafor shows considerable promise in this patient group, but cost-effectiveness and optimum protocols are still to be determined. We present an initial single-centre experience in 40 consecutive PBSC mobilization episodes in which plerixafor was used, in 37 patients. This represents 16% of all mobilization episodes at our centre over this time period (June 2008 to December 2010). Plerixafor was used up-front for one patient, the remaining 36 patients having failed to mobilize adequate PBSC by conventional means. Plerixafor was used with G-CSF but without prior mobilizing chemotherapy in 20 episodes, with the remaining 20 representing “pre-emptive” use of plerixafor to rescue predicted failed mobilization on the basis of poor peripheral CD34+ count on regeneration from mobilizing chemotherapy. A proactive approach to apheresis was adopted, with apheresis always performed after plerixafor administration regardless of peripheral CD34+ count, and a minimum of 3 blood volumes being processed using COBE Spectra® or Spectra Optia® cell separators. Median patient age was 59 (range 3–67); 15 patients were female; diagnoses were myeloma (n=23), NHL (n=5), HL (n=3), primary amyloidosis (n=2), Germ Cell Tumour (n=2), APML (n=1) and neuroblastoma (n=1). The success rate in achieving local institutional minimum CD34+ dose of 2.5 x 10^6/kg for subsequent autologous transplant was 92% (34/37 attempts) at first mobilization using plerixafor, and rose to 100% (3/3 attempts) at 2nd mobilization using plerixafor in the 3 patients failing at first attempt. Median CD34+ dose ultimately achieved was 3.33 x 10^6/kg (2.57–8.42), after a median of 2 aphereses per patient (range 1 to 5) and a mean of 1.73 plerixafor doses per patient (range 1 to 5). Thirty autologous transplants have been carried out on 28 patients (76% autograft rate), with neutrophil and platelet engraftment achieved in all cases (median days to neutrophils>0.5=12; median days to platelets >50=18); one patient received allograft instead due to positive post-treatment PET scan. Compared with our historical failed mobilization rate of approximately 15%, we achieved a 0% failed mobilization rate by using a mean of 1.73 plerixafor doses in 16% of all mobilization episodes, with additional drug cost to the autologous PBSC programme as a whole of £1400 per patient.

P1081

Comparison of T-cell depletion in haplo-identical haematopoietic stem cell transplantation

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Objectives: Haploidentical stem cell transplantation (haplo-HSCT) is an important treatment modality for patients lacking conventional donors. In Switzerland all transplant centers use different transplant protocols and T-cell depletion techniques giving the opportunity to study the effect of

T-cell depletion on the outcome, immune reconstitution and infections after haplo-HSCT.
Methods: Retrospective analysis of all haplo-HSCT in Switzerland since 1998 including pediatric (64%) and adult patients (36%). T-cells were depleted by CD34 positive selection (74%), CD3/CD19 depletion (4%), alemtuzumab in the stem cell product, (11%) or in the conditioning regimen (11%). The groups were categorized in patients with (26%) and without alemtuzumab (74%). The majority of patients received myeloablative conditioning (Cy/TBI ± others, or Bu/Cy ± others), while some patients non-myeloablative (NMA) conditioning with fludarabine and cyclophosphamide.
Results: The study comprised 72 first haplo-HSCT mostly for AML (43%), ALL (39%), or primary immunodeficiencies (10%). The median age at transplant was 13 (0-50) years. 26% of the patients had an early and 64% an advanced disease. 97% had a neutrophil engraftment with a median engraftment time of 14 (0-47) days. Patients with NMA conditioning had a significantly shorter engraftment time (median 2 days, range, 0-9, p<0.001). The one-year overall survival was 45 (±12)%, the cumulative incidence of transplant-related mortality 23 (±11)% and of relapse 55 (±15)%. There were no differences in the OS (p=0.71), TRM (p=0.66) and relapse (p=0.68) in the groups with and without alemtuzumab. T-cell depletion with alemtuzumab caused more profound lymphocyte suppression with a recovery starting 3-6 months after HSCT. Despite extensive antibiotic prophylaxis, most patients experienced infectious complications in the posttransplant period (94%). Bacterial infections (81%) were most frequent after HSCT followed by viral (65%) and fungal infections (31). T-cell depletion with alemtuzumab was associated with a higher cumulative incidence of CMV reactivations at day 100 (44 (±23)% vs 24 (±12)%, p=0.01) in univariate and multivariate analysis adjusted for age, acute GvHD and CMV match.
Conclusions: Haplo-HSCT is a valid option for patients with hematological malignancies without major differences among the different transplant protocols. Patients with alemtuzumab are at higher risk for CMV reactivations.

P1082
Plerixafor (AMD3100) and granulocyte colony-stimulating factor mobilize different cell populations based on immunophenotype and global gene expression signatures
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Rationale: The mechanisms by which AMD3100 and G-CSF alter HSC trafficking are different, suggesting that the final graft with intrinsic properties may be mobilized by these agents.
Aim: To explore differences in cell composition of apheresis products we compared cell mobilized by G-CSF plus AMD3100 combination (Group A) and G-CSF plus chemotherapy (CHT)-standard plan (Group B).
Study design. Twelve patients were mobilized with AMD3100 combination between 2009 and 2010 in Reggio Calabria Hospital, Italy. Four of them did not collected, eight patients underwent apheresis (5 myeloma, 3 lymphoma). A fraction of products was freshly analyzed to determine the cell population and gene expression signature. Leukapheresis products derived from six different patients of group B were used as immunophenotype comparative samples and molecular calibrators (3myeloma, 3 lymphoma).
Methods: Leukapheresis products were analyzed for CD3, CD4, CD8, CD19, CD8/CD56, and CD34 subsets by flow cytometry. Gene expression assays of 144 genes were carried out with TaqMan® Low Density Array Fluidic card. The transcriptome panel included stemness indicators, immune and angiogenic factors involved in cell trafficking. Target gene expression was normalized with expression of similar gene derived from a pool of group B apheresis.
Results: Comparative percentage count of CD4+and CD8+ lymphocytes showed a moderate but no significant increase in apheresis products of group B than group A. Conversely, Plerixafor product had significantly higher percentages of NK and CD19 positive lymphocytes, figure1. These observations were in according to the up-expression level of transcripts for immunoglobulin gene costant region (IGHG3) and lectin-lik NK gene (LLT1), in Plerixafor group, figure 2. Pluripotency genes (Nanog, Oct4, Sox2), self-renewall reporter genes, engraftment indicator genes and angiogenic molecular profile were similar in both groups. No expression difference was determined about CXCR4/CXCL12 axis. Interestingly, the CXCR4 feedback-regulator gene namely as phosphoglycerate kinase 1 (PGK1) was down-expressed in Plerixafor samples.
Conclusion: The number of NK and B cells mobilized by Plerixafor combination was significantly greater than G-CSF + CHT, this was in according with IGHG3 gene expression analysis. It's possible image that the cell composition of autologous transplant graft may influence the early immune recovery and outcome in setting of patients mobilized with AMD3100 scheme.

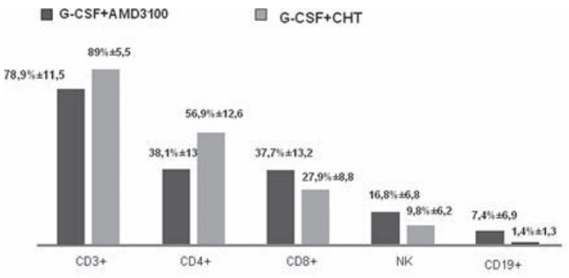

Fig 1. Flow cytometry percentage count of lymphocyte subsets in transplant graft mobilized with different scheme.

	G-CSF+ AM3100	G-CSF+ CHT	
IGHG3 (immunoglobulin a gene costant region)	7,5	1,9	Linea B
LLT1 (lectin-lik NK gene)	3,7	1,3	Linea NK

P1083
Autologous haematopoietic stem cell mobilization with plerixafor and G-CSF: impact of apheresis volume. Data from the Plerixafor European Compassionate Use Study
O. Jaksic, J.F. Apperley, G. Basak, K. Douglas, I. Gabriel, C. Geraldes, K. Hübel, Z. Koristek, F. Lanza, R. Lemoli, G. Mikala, D. Selleslag, N. Worel, I. Bojanic , M. Mohty, R. Duarte on behalf of the European Consortium for Stem Cell Mobilization (ECOSM).

Autologous stem cell transplantation (ASCT) is a standard treatment for a number of hematologic malignancies. Successful ASCT depends on mobilization of CD34+ cells in peripheral blood (PB) and collection by an apheresis procedure. A proportion of patients fail to mobilize and collect an adequate number of CD34+ cells. In order to improve yield, standard apheresis can be repeated daily or each single apheresis extended by processing more blood volumes (BV). Recently, the CXCR4 antagonist plerixafor has showed marked efficacy in mobilizing and collecting CD34+ cells even in a poor mobilizer group. Mobilization by plerixafor is also characterized by different CD34+ cell kinetics in PB. In the present study we included 119 patients who received plerixafor as part of the European compassionate use program and for whom the data of processed BV per apheresis were available. We retrospectively analyzed the impact of apheresis volume on the CD34+ cell yield in this setting. There were

50% males, median age was 57 years. Diagnosis consisted of multiple myeloma (n=50), non-Hodgkin's lymphoma (n=55), Hodgkin's lymphoma (n=12) and other disorders (n=2). In total 66% achieved the primary endpoint of CD34+ >2x10^6/kg. Total number of apheresis performed was 218, with median number of 2 apheresis (range 1-4). Median apheresis volume per patient was 2.74 BV (range 1-6). There were 42 (35%) patients with >3 BV processed per apheresis considered as large volume (LV) apheresis group, while the other patients were assigned to the standard volume (SV) apheresis group. There was no difference between both groups in collecting >2x10^6/kg CD34+ cells, total CD34+ cell yield, number of apheresis days, PB CD34+ count and gender. There was a trend for younger age in LV group (50 vs 54 yr. p=0.07). Consequently, when efficiency of yield per BV processed was analyzed there was a significant difference between LV and SV group (0.60x10^6/kg vs 1.19x10^6/kg CD34+ cells per BV, p<0.003). When each apheresis was analyzed, there was no significant correlation between CD34 yield and BV processed or PB CD34 count. We can conclude that in plerixafor mobilized patients there was no difference between SV and LV apheresis regarding the relevant outcomes with SV being more efficient regarding yield per BV. This may be due to different kinetics of CD34+ cells and importance of other factors in collection procedure. However for definitive conclusions a prospective trial is needed.

P1084

Recurrent cytogenetic abnormalities in ex vivo expanded late passage MSCs from patients with myeloproliferative neoplasms

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Objectives: Myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders including among others, polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Whereas a large body of studies has been performed on hematopoietic cells from patients with MPNs, little is known about the genetics of ex vivo expanded MSCs. The aim of this study was to investigate the genetic asset of MSCs derived from MPN patients at different in vitro culture passages.

Methods: MSCs were isolated and expanded ex vivo, according to standard protocols, from bone marrow (BM) biopsies of 11 patients with MPNs (7 PMF, 1 PV and 3 ET). Five PMF, 3 ET and 1 PV harbored the JAK-2V617F mutation. Genomic characterization of MSCs was obtained through array-comparative genomic hybridization (array-CGH) at both early and late passages (P). Results were compared to those obtained from BM-MSCs of 9 healthy donors (HD).

Results: MSCs of the 11 MPN patients reached senescence at different P (from P6 to P13). We observed that MPN-MSCs became round in morphology during senescence, as compared to HD-MSCs which remained spindle-shaped. Array-CGH analysis showed no chromosomal abnormalities in MPN-MSCs at early P, while MPN-derived MSCs in senescence phase displayed genetic anomalies. In particular, MSCs from the 3 PMF and the only PV patients showed genomic alterations at late P (see Table 1 for details).

MSCs from the 3 ET patients did not exhibit chromosomal abnormalities, even in senescence phase. None of these abnormalities was found in the hematopoietic lineage of the patients; and no correlation was observed between the JAK-2 genotype of the granulocyte and the genetic abnormalities of the MSCs. Array-CGH analysis in HD-MSCs did not show genomic alterations both at early and late P.

Conclusion: MPN-derived MSCs cultured in vitro show cytogenetic abnormalities at senescence which, in PMF-derived MSCs, seem to recurrently affect 7q region, suggesting that a predisposition to DNA damage of 7q could be a peculiar feature of the disease. Further studies are needed to assess whether the genetic abnormalities found in MPN-derived MSCs are acquired at late P or derive from a small clone originally present in the MSCs and expanded throughout the culture. Our results also raise the issue of whether BM-MSCs from MPN patients contain genomic alterations which may contribute to the pathogenesis of the diseases.

Case	Disease	JAK-2V617F (granulocytes)	Passage	Chromosomal abnormalities (Array-CGH)
1#	PMF	Positive	P11	del(7)(pterp22.2) [~4.4Mb] del(7)(p21.3) [~1.2Mb] del(7)(p21.3p15.2) [~14.3Mb] del(7)(p12.3p12.1) [~5Mb] del(7)(q11.22) [~1Mb] dup(7)(q11.23qter) [~ 83.5Mb]
3#	PMF	Positive	P10	1q trisomy [~60Mb]
7#	PMF	Negative	P13	dup(7)(q22.1qter) [~ 60Mb]
8#	PV	Positive	P13	del(1)(pterp34.3) [~35Mb] del(1)(q42.11q44) [~25Mb] del(3)(p21.31p11.1) [~42.5Mb] del(17)(q11.1q11.2) [~350Kb] loss of Y chromosome

P1085

Pre-emptive use of plerixafor in patients mobilizing poorly after chemomobilization: a single-centre experience

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Background: A significant proportion of patients with lymphoid malignancies are hard-to-mobilize with a combination of chemotherapy plus G-CSF (chemomobilization). Plerixafor is a novel drug used to improve mobilization of blood stem cells. However, it has been studied mainly in association with G-CSF.

Methods: We prospectively evaluated the efficacy of 'pre-emptive' use of plerixafor after chemomomobilization in patients who mobilize poorly. During a 15 month period (VIII/2009-X/2010), altogether 63 patients (34 with lymphoma and 29 with myeloma) received chemomobilization and were admitted to our department for stem cell aphaeresis. Patients were considered for pre-emptive use of plerixafor if blood (B) CD34+ counts remained low (usually <10 x 10E6/L) at the time or marrow recovery or if yield of the first collection was poor (<1 x 10E6/kg CD34+ cells).

Results: Sixteen patients (25 %) received plerixafor either due to low B-CD34+counts (median 4 x 10E6/L, range < 1-11) (N=12) or poor yield of the first collection (n=4). Plerixafor was used in 13/34 lymphoma patients (38 %) but only in 3/29 myeloma patients (10%). The median number of plerixafor injections was 1 (0-3). The median B-CD34+ count after the first plerixafor injection was 39 x 10E6/l (< 1-81) with a median increase compared to the pre-plerixafor count of 5 fold. Stem cell aphaereses were performed in 14/16 patients (88 %) receiving pre-emptive plerixafor. With a median of one aphaeresis per patient (0-3), a median of 2.9 x 10E6/kg CD34+ cells were collected (1.6-6.1). All 13 plerixafor-treated patients with grafts > 2 x 10E6/kg CD34+ cells, have received high-dose therapy (BEAM 10, HD-MEL 3) with support of stem cells mobilized with chemotherapy plus plerixafor. All patients have engrafted.

Conclusions: Pre-emptive use of plerixafor after chemomobilization is efficient and safe and should be considered in poor mobilizers to avoid collection failure and need for re-mobilization. Prospective studies are needed to optimize timing and dosing of plerixafor as well as evaluate cost-effectiveness of this approach.

P1086

Use of pre-emptive plerixafor is successful in reducing mobilization failure in myeloma patients undergoing PBSC mobilization with cyclophosphamide and GCSF

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Mobilisation failure is a significant problem for myeloma patients occurring at a rate of 22% in a recent audit at our centre. Although Plerixafor has been successfully used to remobilise patients failing an initial peripheral blood stem cell (PBSC) mobilisation, there is widespread interest in using the agent pre-emptively to prevent failure in patients who are mobilising poorly. To use such an approach efficiently and cost effectively it is essential to establish criteria for intervention. We prospectively analysed the outcome for myeloma patients undergoing PBSC mobilisation with Cyclophosphamide (3gm/m²) and G-CSF (5mcg/kg). Of 34 successfully mobilised patients, 32 had sufficient circulating CD34+ cells to initiate apheresis by day +13 and only 2 patients were successfully mobilised after this time point. As a consequence we have instituted a policy of pre-emptive Plerixafor on day +13 in patients whose PB CD34+ cell count is <10/µl or when the CD34+ cell count had fallen below this level in the presence of an inadequate PBSC harvest (<2 x 10^6/kg CD34+ cells).
Of a total of 46 patients undergoing PBSC mobilisation for myeloma in 2010 we have utilised this approach in 11 (24%); the other 34 patients were mobilised successfully without the addition of Plerixafor. Nine of the 11 patients responded with a median 3.1 fold (range 2.0-5.6) increase in the number of CD34+ cells rising to 25.4/µl (range 2.8-63) after Plerixafor as compared to baseline of 8.1/µl (range 2.6 to 14). Of the 11 patients, 9 collected > 2 x 10^6/kg CD34+ cells and have proceeded to autologous PBSC transplant following high dose Melphalan (200mg/m²) with normal engraftment. The 2 patients who failed pre-emptive Plerixafor had baseline CD34+ levels of <5/µl. In addition 1 of the patients who failed Plerixafor intervention had significant marrow involvement by myeloma and was later successfully mobilised with G-CSF plus Plerixafor following further treatment with PAD chemotherapy. Patients required just 1 (n=7) or 2 (n=4) doses of Plerixafor. This strategy would appear to be effective in reducing PBSC mobilisation failure from 22% to <5%, thus reducing the requirement for costly remobilisation procedures and allowing more myeloma patients to undergo high dose therapy.

P1087

Successful peripheral stem cell mobilization with plerixafor in heavily pre-treated paediatric patients with metastatic medulloblastoma and high-risk neuroblastoma

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Aim: Heavily pretreated pediatric patiens with metastatic medulloblastoma(MB) or high risk neuroblastoma(NB) often fail to harvest enough CD34 + stem cells(SC) to ensure hematopoietic reconstitution, using granulocyte-colony stimulating factor (G-CSF) only. In order to improve SC mobilization in adult patients with non-Hodgkin lymphoma and Multiple Myeloma, CXCR4 inhibitor - Plerixafor was effectively used ,yet the data regarding pediatric patients is scarce. We present the efficacy and toxicity of combining G-CSF with Plerixafor to mobilize SC in five pediatric patients suffering from metastatic MB or high risk NB.
Results: 5 children at a median age of 9 year (range 5-15 year) were included in this study: three patients with metastatic MB and two with high risk NB. All three MB patients were previously treated with craniospinal(CS) irradiation of 36 Gy and 55.8 Gy to the posterior fossa. One patient received additional boost of 9 GY to the sacral area after which he underwent unsuccessful mobilization using G-CSF only (0.3x10^6/kg CD34+ cells). Second mobilization occurred after completing CS irradiation using G-CSF 10 mcg/kg and Plerixafor 0.24 mg/kg. The other two patients with metastatic MB underwent first mobilization after CS irradiation using upfront combination of cyclophosphamide 3 gr/kg and G-CSF 10 mcg/kg combined with Plerixafor 0.24 mg/kg thereafter. The median TNC collected was 23x10^8/kg and CD34+ cells - 20x10^6/kg in two days of apheresis. All three patients underwent autotransplant according to the St. Jude protocol (two patients completed 4 cycles and one child 1 of 4 cycles of transplantation) with median days to neutrophil and platelets engragtment of 10 and 14 days, respectively. Two patients with high risk NB were treated previously with combination of chemotherapy and irradiation (abdominal and bone) and one of them received also high dose chemotherapy and autologous SC support. They failed the first mobilization with G-CSF only (CD34+ cells less than 0.6x10^6/kg). The second mobilization included G-CSF and Plerixafor and the median TNC collected were 20x10^8/kg and CD34+ cells - 4.5x10^6/kg in 1 day of apheresis. Side effects were documented in two of the five patients who received cyclophosphamide and included abdominal pain, diarrhea and vomiting.
Conclusions: Plerixafor and G-CSF is feasible in pediatric patients enabling SC harvest in heavily pretreated patients. Further study in large cohort of pediatric patients is warranted.

P1088

Successfull mobilization of peripheral blood stem cells in lymphomas with pegfilgrastim following cisplatin-aracytin-containing regimen

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Background: The combination of chemotherapy and granulocyte colony-stimulating factors (G-CSF) increases the CD34+ yield compared with growth factors alone. Respect to the unconjugated form of G-CSF, the pegylation of filgrastim leads to the prolongation of its half-life due to decreased renal elimination. Single-dose of Pegfilgrastim has similar effects in the prophylaxis of chemotherapy-induced neutropenia as the daily filgrastim and the efficacy in peripheral blood stem cells (PBSC) mobilization needs to be confirmed.
Methods: From January 2006 to October 2010 80 patients (pts) with lymphomas were candidate to stem cell mobilization and high-dose chemotherapy. The vast majority of pts (64) were relapsed or in progressive disease while 16 pts were newly diagnosed. They received in median one previous line of therapy (range 1-4) and the median age at collection was 48 years (range 19-74). As mobilizing chemotherapy we used a regimen containing cisplatin and aracytin (modified ESHAP), followed by 6 mg subcutaneous single dose of Pegfilgrastim 24 hours after the end of treatment. Peripheral CD34+ cell evaluation started from the increase of WBC above 1x10^9/L. Using a Cobe Spectra separator, the harvest started when the number of circulating CD34+ cells was >10µL and performed daily until target number of PBSC ≥2x10^6 was reached.
Results: Seventy-five pts (94%) performed successfully PBSC collection and the target yield was obtained with one leukapheresis cycle in 57 (76%) pts. Of note, in pts who failed to mobilize (6%), one previously underwent high-dose radioimmunotherapy and one autologous stem cell transplant.
In 85% of cases the peak of peripheral CD34+ was observed in days 8-10 (range 7-12 days). At the time of harvest the median peak of absolute CD34+ cells in the peripheral blood was 85 µL (range 7-578) and the median number of collected CD34+ cells/Kg was 9,4x10^6 (range 1,36-64). Fifty-six pts obtained an "optimal" harvest (>5,0x10^6/Kg).
Conclusion: Our experience shows that a single injection of Pegfilgrastim following cytotoxic chemotherapy containing cisplatin and aracytin is a valid approach capable of mobilizing

sufficient number of PBSC in lymphoma pts with timing of apheresis sessions widely predictable. Besides a high percentage of pts (76%) obtained an optimal collection with just a single PBSC collection. Moreover the single injection of Pegfilgrastim resulted feasible and well tolerated in the out-patient management.

P1089

CD34+-selected stem cell boost for poor graft function after allogeneic haematopoietic stem cell transplantation

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About 5–27% of patients (pts) experience a poor graft function (PGF) defined as hemoglobin (Hb) <10.0 g/dL and/or neutrophils (Neu) <1,500/µL and/or plateletes (Plt) <30,000/µL with complete donor chimerism on day 30 after HSCT. This complication is associated with considerable morbidity and mortality due to infections and hemorrhagic complications. Some reports showed the potential of CD34+-selected stem cell boost (SCB) in this setting. However, this approach might also exacerbate GvHD. To further assess the safety and efficacy of this approach, 28 patients (m/21, f/7; median age, 49 y; 34 – 64) with PGF received SCB without conditioning at University Medical Center Hamburg and at Institut Paoli Calmettes, Marseille. Pts were transplanted (related, n=11; unrelated: matched, n=12; mismatched, n=5) initially for myelofibrosis (n=11), AML/MDS (n=3), CML (n=3), ALL (n=2), NHL (n=3), multiple myeloma (n=2), or aplastic anemia (n=4).
At the time of the SCB (median 5 mo (1-228) post-transplant) the median numbers of Neu and Plt for evaluable pts were 945/mkl (840 – 4,160) and 16,000/mkl (10,000 – 94,000), respectively. The 17/25 (68%) pts required red blood (n=10), platelet (n=1) transfusions or both (n=6); 22/28 (79%) pts experienced PGF in 2, while 6 (21%) in 3 hematopoietic lines.
The pts received a median of $3.3x10^6$ CD34+/kg bw (1-14) and of $8x10^3$ CD3+/kg bw (2-70). All SCBs were performed by MicroBeads-based cell selection (Miltenyi Biotec).
At a median follow-up of 25 mo (10-107) from SCB 11 pts (39%) had expired (relapse, n=3; GvHD, n=4; severe infection, n=3; severe hemorrhage, n=1). GvHD after SCB developed in 6/28 pts (acute, 21%) and 8/27 pts (chronic, 30%), respectively. Of those, 4 pts already had GvHD after HSCT. We have not observed any significant correlations between the GvHD rate and CD3+ cell count. Also, there was no significant difference in GvHD rates between pts who received SCB before a median of 5 mo (6/11, 55%) and after it (8/17, 47%).
The 20/24 evaluable pts (83%) resolved from PGF at day 30 after SCB (25% increase in Neu/Plt counts and/or transfusion-independence). Of 17 alive pts, 12 (71%) were in CR, 4 (24%) in PR, and 1 (5%) had progressive disease. The 2-year overall survival after SCB for all 28 pts was 60%.
In conclusion, SCB represents an effective strategy to improve the function of graft in cases of PGF, however, despite the T-depleted approach, there is still a risk of GvHD.

P1090

Experience of autologous peripheral blood progenitor cell mobilization with biosimilar granulocyte colony-stimulating factor: a single-centre, prospective-historical control study

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Objectives: Biosimilars are similar but non-identical versions of existing biopharmaceuticals, with regulatory approval being provided on the basis of comparable quality, safety and efficacy to a reference product. Recently, biosimilar versions of granulocyte colony-stimulating factor (G-CSF) have become available. Here we report the first comparative data of a biosimilar G-CSF and its reference product for autologous peripheral blood stem cell (PBSC) mobilisation and collection.
Methods: A total of 40 consecutive patients with a haematological malignancy scheduled to receive biosimilar G-CSF (Zarzio®) following first-cycle chemotherapy for treatment and autologous PBSC mobilisation were prospectively included at a single centre between March and September 2010. These patients were compared with a matched historical control group who had been treated with G-CSF (Neupogen®) at the same centre between 2007 and 2009. Patients in both groups were treated according to the same clinical protocol with chemotherapy administered on day 1 and G-CSF administered at a dose of 5 ug/kg/day from day 9 (lymphoma) or 10 ug/kg/day from day 16 (multiple myeloma) until white blood cell (WBC) recovery. PBSC harvesting was considered successful if at least $3×10^6$ CD34+ cells/kg were collected. If three consecutive CD34+ tests were below 10 uL then PBSC harvesting was not performed.
Results: Patients characteristics were similar in both groups with no significant differences in median age, diagnosis, number of previous chemotherapy courses or chemotherapy mobilisation regimen. In both groups, five G-CSF injections (median) were required to complete PBSC mobilisation. Median pre-leukapheresis peripheral blood WBC counts (31 x 10^9/L vs 28.2 x 10^9/L; p=0.68) and CD34+ counts (55.5 vs 60.0 ul; p=0.71) were similar in the Zarzio® and Neupogen® groups. The median number of CD34+ cells collected in the first leukapheresis was also similar with Zarzio® and Neupogen® (5.5/kg vs 4.5/kg; p=0.26). The number of leukaphereses necessary to harvest the minimum CD34+ cell count (3 x 10^6/kg) was the same in both groups (median 1, range 1–3). PBSC mobilisation failed in just three patients in the Zarzio® group (7.5%) and one patient in the Neupogen group (2.4%) (p=0.36).
Conclusion: Biosimilar G-CSF (Zarzio®) is comparable to Neupogen® for PBSC mobilisation and collection after chemotherapy and so may provide a more cost-effective strategy.

P1091

Successful mobilization of peripheral blood stem cells in a healthy volunteer donor by addition of plerixafor after failure of mobilization with G-CSF alone

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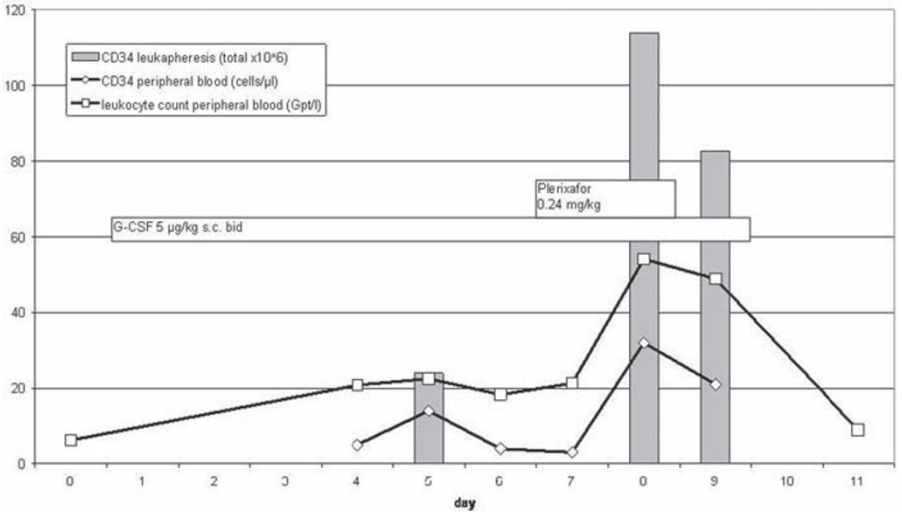
A 50 year old woman was chosen as sibling donor for peripheral blood stem cells for her 46-year old brother. We started mobilisation with G-CSF (5µg/kg s.c. 2xdaily) 5 days before leukapheresis. At time of first leukapheresis we found an insufficient mobilisation of CD34-positive stem cells into the peripheral blood, the CD34 count was 14/µl. We were unable to collect a sufficient amount of CD34-positive stem cells (yield: 23.9 x 106 CD34+ cells, equivalent to 0.32 x 106 CD34+ /kg body weight of the recipient).
In spite of continued application of G-CSF the CD34 count decreased to 4/µl and 3/µl at the next 2 days. A marrow harvest was refused by the donor and initiation of a search for an unrelated donor was no option. The harvested cells were grafted.
As a consequence we discussed a combined mobilisation with G-CSF and Plerixafor. The donor agreed after detailed explanation of risks. After 6 days of ongoing stimulation with G-CSF we added plerixafor in recommended dose of 0.24 mg/kg body weight/day. On the next days CD34 count in peripheral blood raised to 32/µl and 21/µl respectively. The peripheral CD34 cell count increased satisfyingly under plerixafor despite falling counts under ongoing G-CSF alone. The leukapheresis performed at the next 2 days after initiation of plerixafor yielded 113.9 x 106 and 83.6 x 106 CD34+ cells, equivalent to 1.50 x 106 and 1.09 x 106 CD34+ cells per kg body weight of the recipient.

[P1091]



At the end the addition of plerixafor to the ongoing stimulation with G-CSF enabled us to collect an adequate number of CD34+ cells (total 2.9 x 106 CD34+ cells per kg body weight of the recipient) for allogeneic transplantation. The recipient did well and engrafted in time (leukocyte count > 1.0 Gpt/l on day +13, thrombocytes >20 Gpt/l on day +16). The patient was discharged on day +35. At last follow-up on day +263 the myeloma was in complete remission with full donor chimerism.
The use of plerixafor in a healthy donor is an off-label use, however, there was no alternative. Donor's follow up is actually 8 months without any serious side effects. Blood smear normalized within 3 days. Further visits of the donor were arranged to register possible long-term effects. Although our approach is no standard policy in case of G-CSF failure, the addition of plerixafor to an ongoing stimulation with G-CSF may be considered as a rescue procedure in healthy related donors ineligible for conventional bone marrow harvest after informed consent.

P1092
Comparison of efficacy and safety of biosimilar granulocyte-colon stimulating factor (Zarzio®) to recombinant granulocyte-colony stimulating factor- filgrastim (Neupogen®) in haematopoietic stem cell mobilization
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Recombinant granulocyte colony-stimulating factor - filgrastim (Neupogen®) has been widely used for many years to mobilize hematopoietic stem cell. We investigated efficacy and safety of a new biosimilar G-CSF- (Zarzio®) in comparison to filgrastim (Neupogen®) in hematopoietic stem cell mobilization in patients with haematological malignancies. Forty-eight patients (21 with multiple myeloma, 16 with non- Hodgkin's lymphoma, 8 with Hodgkin's lymphoma, and 3 with other diagnosis) qualified to the peripheral stem cell mobilization were included in the study. Median age of the patients was 50.5 years (range 19–63) and the male/female distribution 26/22. Median time from diagnosis to mobilization was 10.5 months in whole group. After administration of mobilizing regimens, patients were randomized to receive treatment with Zarzio (24 pts) or Neupogen (24 pts) in a standard daily dose 10 µg/kg. The median number of the days of G-CSFs administration was similar in both patient groups. There was no statistically significant differences in the number of mobilized CD34+ cells /µL in peripheral blood and the number of CD34+ cells/ kg body weight between two groups. The adverse event profile was similar. Comparison of the efficacy of Zarzio and Neupogen in hematopoietic stem cell mobilization is presented in Table 1. Zarzio demonstrated similar efficacy and safety profile as the reference medication filgrastim (Neupogen®) in hematopoietic stem cell mobilization in patients with haematological malignancies. In conclusion, the use of Zarzio in the mobilization of hematopoietic stem cells in patients with hematological malignancies was safe, and resulted in a sufficient stem cell harvest in the majority of patients.

Table 1. Comparison of the efficacy of Zarzio and Neupogen in hematopoietic stem cell mobilization

	Number of G-CSF days /mean ± SD/	Number of CD34+ cells/µL in peripheral blood /median, range/	Number of CD34+ cells x 10^6 / kg b.w. /median, range/	Number of apheresis /mean ± SD/
Zarzio group	7.5 ± 2.6	89 (3.4-900)	9.9 (0-40)	1.37 ± 0.87
Neupogen group	8.6 ± 2.1	68 (10-323)	11.1 (2.7-48)	1.58 ± 0.65

P1093
Depletion of TcRab+ and CD19-positive cells from leukapheresis products with the CliniMACS device
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Depletion of TcRab+ T cells can prevent GvHD after allogeneic stem cell transplantation from HLA non identical donors. In contrast to depletion of CD3+ T cells valuable TcRgd+ T cells are spared. We present our results using the CliniMACS device for depletion of apheresis products intended for allogeneic transplantation.
Apheresis cells were subsequently incubated with TcRab+-Biotin reagent and CliniMACS anti-biotin reagent/Clinimacs CD19 reagent and cells were than separated by the CliniMACS device using the DTS tubing set and program Depletion 3.1.
17 separations were performed. Apheresis products with 5.6 ± 2.5 x10e10 MNC were used with a mean of 421 ± 280 x 10e6 CD34+ cells. TcRab+ cell content was 27.5 ± 13.2 percent and B-cells were 5.2 ± 1.7 percent in the apheresis product. After depletion, a mean of 0.0028 ± 0.0046 percent of TcRab+ T cell could be detected and B cells, determined as CD20+ cells were reduced to 0.015 ± 0.019 percent. By this TcRab+ cell were depleted by log 4.48 ± 0.51 and B-cells by log 4.1 ± 0.5. Recovery of mononuclear cells was 51.6 ± 9.1 percent and recovery

of CD34+ cells was 71.9 ± 11.3 percent. Recovery of CD56+ NK-cells was 77.6 ± 24.2 percent. Viability of cells was 97.8 ± 1.4 percent after separation.
Flow cytometry: Viable TcRab+ cells were defined by their cell scatter characteristics of lymphocytes, positivity for CD3 and TcRab and negativity for propidium iodide staining and TcRgd, respectively. Furthermore, a sample of depleted cells was spiked with about 3 percent of cells from the positive fraction and the gate for TcRα-β cells was set using the dot plot of this sample. A minimum of 1 x 10e6 events was acquired for analysis.
Due to reduced intensity of CD19 antibody after incubation with CD19 reagent, CD20 was used to determine the amount of residual B cells after depletion.
Profound depletion of TcRab+ T cells could be achieved with the CliniMACS system and DTS tubing set comparable to results after CD34 enrichment. Recovery of stem cells was slightly better than that of CD3/CD19 depletion. Detection of residual TcRab+ and B cells by flow cytometry is demanding and needs special attention.

P1094
Mobilization of CD34+ cells in the course of PBPC collections: does it exist?
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Objectives: The results od PBPC transplantation are affected by mobilization of progenitor cells, timing of collections, and by the use of optimum collection technique. Previously, we observed that the yield of CD 34+ cells was higher in large volume leukapheresis (LVL) than in the standard collections. Statistical analysis proved that the yield of CD 34 was related only to the precollection CD 34+ cells concentration in blood. The cause of better results from LVL remained still unexplained. Therefore we tried to verify if any process of mobilization of CD 34+ cells does exist in the course of standard and LVL collections.
Methods: The results of 41 first PBPC collections in 41 patients (15 women/26 men) with hematooncological diseases were evaluated. The group of 25 patients was directed to the LVL, while another group of 16 patients was collected in the standard regimen. In the standard collections 2,3 (2-2,9) total blood volumes /TBV/of the patients were processed, while in LVL 3,8 (3-4,2) TBV were procesed (Cobe Spectra, Caridian).
Results: In patients collected by the use of standard regimen, precollection CD 34+ concentration in blood was 72 (20-277) [103/ml], and in patients collected using LVL the concentration of CD 34+ cells was 81 (20-626) cells in blood [103/ml]. The yield of CD 34+ cells from the standard collections was 6 (1-17), while from LVL 11 (2-33)[106/kg b.w.]. Percentage of CD 34+ cells collected “from blood to bag” was 120 (42-1568) in standard collections, and 177 (65-1052) in LVL. Percentage of leukocytes collected “from blood to bag” was 26 (15-88) in standard, and 31 (14-102) in LVL. Percentage of platelets collected “from blood to bag” was low; in standard procedures 4 (3-7), and in LVL 5 (3-8).
Conclusion: Mobilization of CD 34+ cells was proved in the course of Standard and LVL. In LVL, the percentage of mobilized cells was higher than in the standard collections. This observation may correspond with higher yield of CD 34+ cells from LVL. On the other hand any mobilization of leukocytes and platelets was not observed. Although the release of progenitor cells from bone marrow to blood in the course of collections has not yet been explained, it seems to be useful to study it in future. The process may affect the efficiency of apheresis, and its understanding could help to optimize of PBPC collection technique.

P1095
Effect of energy shock wave treatment on cord blood CD34+ cell adhesive properties: role of hERG1 channels
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Objectives: We previously showed that extracorporeal shock waves (ESW) improve cord blood (CB) CD34+ cell in vitro expansion and engraftment in NOD/SCID mice. Here, we investigated the effect of ESW on various activities involved in homing mechanisms of CB hemopoietic progenitors/stem cells (HPCs/SCs). Since the relevant role of ion channels in cell adhesion, we tested the effect of ESW treatment on the expression of hERG1 (ether-a-go-go-related gene) potassium channels in CB CD34+ cells.
Methods: CB CD34+ cell selection: by Miltenyi MiniMACS. Flow cytometry: CD34, CD49d, CD49e, CD11a, CD44, CD62L, CD31, CD184 (CXCR4) expression was evaluated in two-colour fluorescence. Cell cycle analysis of CD34+ cells by Coulter DNA PREP kit. Cell adhesion assay: onto fibronectin-coates wells. Cell migration assay: transwells coated with fibronectin through SDF1 gradient. CD34+ expansion: in IMDM+10% FCS and SCF 50 ng/ml+flt3 ligand 50 ng/ml+ TPO 20 ng/ml+ IL6 10 ng/ml for 7 and 14 days. Shock wave treatment: by a piezoelectric device generating a energy flux density of 0.32 mJ/mm2 per 500 shots. hERG1 expression: quantitative RT PCR and by flow cytometry with an anti hERG1 MoAb. Patch-clamp recordings: hERG currents were recorded by an Axopatch 1D (Axon Instruments).
Results: After ESW exposure, no significant variations in the expression and fluorescence intensity of CD34+ CAM and CXCR4 was observed, however CD34+ adherence significantly increased (32.3±11% versus 8.1±7.7% in controls). This increase was maintained also after 7 and 14 day in vitro expansion. No significant differences were observed in cell migration

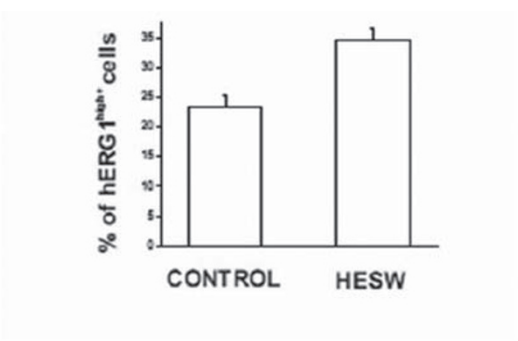
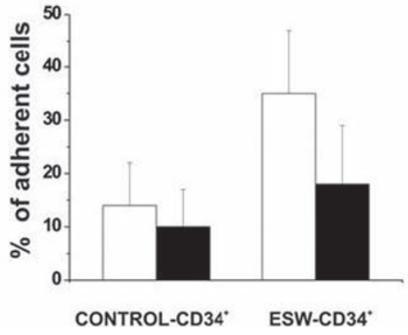


Fig1 Percentage (mean ± SEM) of hERG1^high+ cells in control-CD34+ and ESW-CD34+. hERG1 was considered overexpressed when the fluorescence value is higher than 8000.(ESW-CD34+ vs control CD34+ p<0.005)



and cell cycle distribution. The percentage of hERG1^{high}+ CD34+ cells was significantly increased. In patch-clamp experiment, IhERG was recorded in CD34+ cells exposed to ESW, while barely detectable in CD34+ control cells. ESW treated-CD34+ cells were significantly more hyperpolarized than controls. Addition of the hERG1 inhibitor Way 123,398 caused a sudden depolarization of the treated cell, whereas it had no effect on control cells. In further experiments on ESW-treated cells, the adherent fraction was 35±14% versus 18±11% in the presence of Way 123,398.
Conclusions: We evidenced a novel mechanism which leads to an increase of the adhesive properties of CB CD34+ cells. Such mechanism is triggered by ESW treatment and occurs with a strong involvement of hERG1 channels. We propose the pretreatment with ESW as a new method to manipulate the HPCs/SCs.

P1096

Haematopoietic stem cell mobilization with plerixafor: the University Central Hospital of Asturias experience, Spain

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Background: CD34+ cell dose has been showed to be a strong predictor for the stem cell transplantation (SCT) success.
Objective: to analyze the safety and the effectiveness of plerixafor in poor mobilizers with lymphoma and multiple myeloma.
Patients and method: during the period September'08-November'10 we used plerixafor in 20 mobilization procedures (in 18 pts: 11 male, 7 female). Median age was 56 years (range: 22-69). The pts were diagnosed with MM (44%), NHL (33%) and HL (22%). Median of previous lines of therapy was 3.5 (range: 1-7). Seven pts (39%) had been treated with radiotherapy and/or lenalidomide, and 6 (33%) had undergone prior auto-SCT. The indications for the use of plerixafor were: a) previous mobilization failure (6 cases), b) less than 5 circulating CD34+ cells/µ;L at the start of apheresis (7 cases), c) low amount of CD34+ cells collected during the initial apheresis (6 cases), and d) prediction of poor mobilization (in 1 pt with 4 lines of therapy, included radiotherapy and lenalidomide). The goal of the collection was: a) ≥ 2x10^6 CD34+ cells/kg for auto-SCT (12 pts); b) ≥ 1x106 CD34+ cells/kg for back-up for unrelated donor-SCT (URD-SCT)(6 pts).
Results: the pts received G-CSF at 20 mg/kg/day for 4 consecutive days. On the evening of day/s 4 and/or 5, plerixafor at 0.24 mg/kg/day s.c. was administered, and apheresis was initiated approximately 10 h later. Daily administration of G-CSF and plerixafor continued until sufficient cells had been collected or the investigator decided that the patient had failed to mobilize. The target number of cells was reached in 15 pts (83 %), after a median of 1 apheresis procedure (range: 1-5). A median of 1 injection of plerixafor (range: 1-3) was necessary. Up to the last follow-up, 8 pts had proceeded to auto-SCT. The median dose of CD34+ cells infused was 2.23x10^6/kg (range: 2-3). Engraftment was achieved in 100 % patients. Median time to reach neutrophils > 1.500/µL was 13 days (range: 12-17) and platelet > 100.000/µL 25 days (range: 20-270). Two pts had mild diarrhea the evening of the plerixafor administration. No local reactions were documented.
Conclusions: a) Plerixafor combined with G-CSF for SC mobilization had an excellent safety profile; b) Plerixafor plus G-CSF was effective to obtained enough CD34+ cells for auto-SCT and back-up for URD-SCT in a selected population of poor mobilizers.

P1097

Efficacyand safety of mobilization with etoposide and granulocyte-colony stimulating factor in heavily pre-treated and/or poorly mobilized patients

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Introduction: Peripheral blood stem cells are currently the source of choice for autologous stem cell transplantation. Optimizing peripheral blood stem cell collection remains an important goal especially in heavily pretreated patients. Etoposide plus granulocyte colony stimulating factor is a regimen for mobilization preferred in recent years. Here, we report our own experience with this regimen in various hematological malignancies.
Methods: Heavily pretreated and/or poorly mobilized 20 patients were included in the study. Median age was 38 years (range 20-62). 9 patients were female and 11 patients were male. Etoposide was given at a dose of 250 mg/m2/12 hours intravenously on days 1, 2 and 3. Granulocyte colony stimulating factor (G-CSF) (filgrastim 5 µg/kg once daily) was started on day 4 through the final day of collection. Collection was started when the peripheral blood CD34+ cell count was ≥ 10/µl. The target total CD34+ cell count was minimum 2x106/kg and poor mobilizers were defined as patients unable to collect this target.
Results: Diagnosis were: non Hodgkin's lymphoma (n=9), Hodgkin's lymphoma (n=5), acute myeloid leukemia (n=5) and multiple plasmositoma (n=1). Median courses of chemotherapy received before mobilization was 5.5 (range 3-12). 3 patients also received radiotherapy. The median time between last chemotherapy and mobilization was 2.5 months (range 1-14). Febrile neutropenia was observed in 11 of 20 patients. Minimum hemoglobin was 6 g/dl, minimum white blood cell count was 0.26 x10^3/µl and minimum platelet count was 4x10^3/µl. Maximum white blood cell count was 64x10^3/µl and was reached on day 18. 17 of 20 patients reached minimum target total CD34+ cell count. Total CD34+ cell count was >5x10^6/kg in 7 patients. 2 patients needed 1, 5 patients needed 2, 6 patients needed 3 and 4 patients needed 4 days to collect these numbers. Only 2 of 7 patients poorly mobilized with prior mobilization regimens were poorly mobilized again with this regimen.
Conclusion: Etoposide and G-CSF is an effective and safe mobilization regimen for heavily pretreated patients and can be an option for poor mobilizers after standard mobilization regimens.

P1098

Harvesting of mobilized peripheral blood stem cells alters the plasma cytokine profile in patients with multiple myeloma

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Background: Therapeutic interventions may cause host responses that affect the residual disease. Cancer cell dormancy can be defined as in vivo long-term persistence of occult cancer cells, and it has been hypothesized that systemic cytokine responses can trigger progression of residual disease, e.g. through initiation of angiogenesis. This may be true also for B lymphocyte malignancies.
Methods: Plasma samples were collected from 15 consecutive multiple myeloma patients undergoing peripheral blood stem cell harvesting following mobilization with chemotherapy and granulocyte colony-stimulating factor. Patients were compared with healthy blood donors undergoing platelet apheresis. We investigated the systemic levels of 34 different cytokines.
Results: Leukapheresis altered the systemic cytokine profile for the myeloma patients. Hepatocyte growth factor (HGF) is a cytokine that function as a growth factor for myeloma cells and in addition has proangiogenic effects, and especially the levels

of this factor were altered following apheresis. HGF levels were increased prior to stem cell harvesting compared with levels in healthy individuals; and a further increase was detected immediately after apheresis. HGF levels determined 24 hours after apheresis did not differ from preapheresis levels. Relatively high HGF levels were also detected in graft supernatants. Preapheresis levels of angiopoietin-2 and vascular endothelial growth factor (VEGF) were increased in the patients whereas angiopoietin-1, angiogenin and basic fibroblast growth factor levels did not differ from healthy controls. Stem cell harvesting decreased angiopoietin-1 and VEGF levels but did not alter the other mediators. Plasma levels of the microvascular endothelial cell marker endocan also increased during leukapheresis.
Conclusion: Autologous stem cell harvesting alters the systemic cytokine profile (especially HGF levels) and plasma endocan levels in myeloma patients.

P1099

Plerixafor as mobilizing agent for patients failing a previous mobilization attempt and as first-line mobilizing therapy in patients affected by multiple myeloma

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Peripheral blood stem cells (PBSC) represent a well established viable alternative to bone marrow as a source of hematopoietic stem and progenitor cells for autologous transplantation, a standard approach for treatment of different hematological malignancies. A dose of $2.0x10^6$ CD34+cells/kg is generally considered sufficient to allow a successful engraftment after high-dose chemotherapy. Nonetheless a high fraction of patients (pts) ranging from 11 to 53% will fail mobilization of stem cells and won't be able to benefit from autologous stem cell transplantation (ASCT). Plerixafor (AMD3100) a bicyclam antagonist of the SDF-1 α/CXCR4 complex, has been previously reported to improve PBSC collection in pts undergoing PBSC mobilization. From April 2009 to November 2010, a total of 13 pts affected by hematological malignancies (5 Hodgkin Lymphoma, 5 Non-Hodgking Lymphoma, 3 Multiple Myeloma) who had already failed a previous mobilizing attempt, underwent stimulation with plerixafor at a standard dose after receiving G-CSF for 4 days in order to mobilize PBSC; other 3 patients who had never been mobilized before, received the same stimulation therapy following immunomodulating drugs containing induction chemotherapy for multiple myeloma (MM). Pts characteristics were the following: 12 were female, 4 were male, median age was 53 years (27-70); median number of previous lines of therapy was 2 (1-7) and 4 pts had received radiotherapy. Overall plerixafor administration was safe and no serious adverse events were reported. The median number of circulating CD34+ cells/microL following plerixafor was 22 (11-122). All 16 patients were able to collect the minimum required dose for ASCT in a median number of procedures of 1 (1-3); median numbers of CD34+ cells collected was $2.5x10^6$/kg; notably the 3 patients affected by MM, stimulated with plerixafor upfront were able to collect in a single procedure the target CD34+ cell dose to be used for a tandem transplant (7.7, 8.4 and $10x10^6$ cells/kg, respectively). At the time of the analysis, 13 of the 16 pts had already undergone ASCT: 13/13 engrafted with a median time to ANC≥500/ul of 12 days and to a PLT≥20000 of 16 days. We conclude that mobilization with plerixafor is safe and effective being able to rescue patients who failed a previous mobilizing attempt and it might represent an effective mobilization strategy for pts with MM who need to collect a greater number of CD34+ cells/kg for a tandem transplant.

P1100

European paediatric experience with plerixafor for autologous PBSC mobilization from children failing to mobilize PBSC by conventional means: an initial series of 6 patients from 2 centres

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Although plerixafor shows considerable promise as a novel agent for PBSC mobilization, experience in children has so far been limited. We present an initial paediatric experience of 8 mobilization attempts using plerixafor in 6 patients from two European centres, all of whom ultimately mobilized a transplantable PBSC dose after one (n=4) or two (n=2) mobilization episodes incorporating plerixafor. Two mobilization episodes consisted of delayed re-mobilization with G-CSF plus plerixafor due to previous failed chemotherapy-based mobilization; in the remaining 6 episodes, plerixafor was used on a "pre-emptive" basis to rescue predicted failed mobilization on the basis of poor peripheral CD34+ counts on regeneration post-mobilising chemotherapy. Median age was 8.5 years (range 3 months – 16 years). Diagnoses were NHL (n=2), neuroblastoma (n=1), medulloblastoma (n=1), Ewing's sarcoma (n=1) and PNET (n=1). One patient failed to undergo apheresis at first mobilization attempt using plerixafor, with peak peripheral CD34+ count of just 3 per microlitre after 2 plerixafor doses. The remaining 7 mobilisation attempts all yielded some PBSC, with median peak peripheral CD34+ count of 34 per microlitre (range 12-173) and a median CD34+ dose of 3.69 (range 0.39–9.32) being achieved after a median of 2 aphereses (range 1-5). All 6 patients ultimately achieved a transplantable CD34+ dose (median total dose 5.34×10^6/kg; range 2.69–9.32). Plerixafor toxicities were all minor and self-limiting (insomnia – 2 patients; bone pain – 1 patient; arthralgia – 1 patient). Three patients have undergone autologous PBSC transplant: all achieved neutrophil and platelet engraftment, with median of 11 days to neutrophils > 0.5. Of the transplanted patients, one is alive without disease progression at 8.5 months post-transplant; one died of progressive NHL at 6.5 months post-transplant; one died of Pneumocystis pneumonia at 3.5 months post-transplant. Three patients have not yet undergone transplant: one due to poor prognosis and co-morbidities (still alive at 8.5 months post-plerixafor but with disease progression at 7.5 months); one due to disease progression (alive at 11 months post-plerixafor but with progressive disease); one patient currently awaiting transplant at 1 month post-plerixafor. In our initial experience, plerixafor is highly effective in PBSC mobilization from children requiring autologous HSCT failing to mobilize by conventional means, with acceptably low toxicity.

P1101

Harvest of peripheral blood stem cell from patients with haematological malignancies is associated with a clinically significant reduction in platelets counts

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The collection of PBSC from healthy donors after GCSF mobilization is associated with reduction in platelets counts. The magnitude of this effect, in patients with hematological malignancies undergoing PBCS mobilization and harvest is not known. The purpose of this study was to explore the effect of platelet reduction after PBSC collection in this population.
Materials and methods: We analyzed 218 collection procedures of GSCF mobilized PBSC of 157 individuals, of whom 100 were performed on 82 healthy donors and 118 on 75 patients. Among patients, 30.1% had NHL, 24% had Multiple Myeloma, 12% had Hodgkin's lymphoma and 33% had other malignancies. All collections were done using Cobe Spectra™ apheresis system. Healthy donors were harvested after 4 days of GCSF

treatment, while patients, mobilized with chemotherapy and GCSF, were harvested when target CD34+ cells reached ≥ 10 microliter. Platelet counts were obtained before and immediately after the procedure.
Results: Overall a median decrease in platelet count of 52 *10^9/L across all collections was documented. Linear regression analysis revealed that 70% of the decrease variance is explained by the baseline platelet count. A higher baseline count among healthy donors was noted (mean 220*10^9/L, SD 83*10^9/L), compared to patients (mean 117*10^9/L, SD 80*10^9/L). Hence, the absolute decrease in platelet counts among healthy donors was 100*10^9/L (SD 50*10^9), and only 47*10^9/L for ($p<0.0001$). However, clinically significant reduction in platelet count (defined arbitrarily as platelet count of less than 50*10^9/L) was found in 45 collections from 26 autologous donors (34.6%), and in only one collection from a healthy donor. Notably, higher reduction in platelets was accompanied by higher platelet count in the collection bag (rp= 0.778).
Conclusions: Reduction in platelet count after PBSC harvest occurs almost universally. A clinically significant reduction to an absolute platelet count of less than 50*10^9/L was noted in more than one third of patients with hematological malignancies. This reduction was not associated with clinically apparent bleeding in this cohort. Additional studies are needed to determine the bleeding risk from this procedure, and the delay of future therapy courses due to thrombocytopenia.

P1102
Measurement of aldehyd dehydrogenase activity in allogeneic or autologous peripheral stem cell grafts may substitute time- consuming cell cultures
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Background: Autologous and allogeneic stem cell (PBSC) transplantation are a curative option in hematological-oncological diseases.
In daily routine stem cell harvests are measured on CD34 antigen and vitality. The colony-forming ability (CFU) of the harvested stem cells is predictive for the hematological engraftment but results are not available until 14 days. The metabolic marker aldehyd dehydrogenase (ALDH) was found in hematological vital stem cells. Former investigations described a correlation between ALDH activity of harvested stem cells and CFU. Therefore this assay may be useful for characterizing PBSC graft quality, especially as the results are available simultaneously to the CD34 and vitality data.
In a prospective analysis we investigated the ALDH activity in freshly collected PBSC and in frozen products and correlated the results with the number of CFUs
Material and methods: In the allogeneic setting 16 PBSC harvests from 13 donors, 7 males and 6 females, median age of 45 years (24-60) and in the autologous setting 42 products of 25 patients, 15 males and 10 females, median age 52 years (21-67) were investigated.
Stem cell harvests were measured on CD34+ cells and vitality with 7 AAD by flow cytometry according to the ISHAGE protocol. ALDH activity was also determined by FACS analysis using the Aldeflour®kit (company) according to the manufacture's instructions.
Analyses were done in freshly collected PBSCs and in cryopreserved products before transplantation or after 6 months of storage, respectively.
Results: The allogeneic stem cell grafts had a CD34 purity of 0.74% (0.47-1.83), an ALDH activity of 0.7% (0.45-2.18) and a CFU content of 236 x 10^5 (28-480).
The autologous grafts showed a CD34 purity of 0.6% (0.11-7.63), an ALDH activity of 0.59% (0.15-8.89) and CFUs of 139 x 10^5 (0-892).
CFUs and ALDH activity showed a higher correlation than CFUs and CD34+ cells in the autologous grafts (R = 0.73 vs R = 0.40) and no difference in the allogeneic grafts. Low initial ALDH activity (below 0.20%) was combined with low numbers and low recovery of CFUs after thawing frozen PBSC. No correlation was observed between vitality and CFUs in both settings.
Conclusion: Measuring of ALDH activity in PBSCs may alleviate the PBSC harvest management in patients with CD34 yields near the threshold for successful transplantation.

P1103
Haematologic recovery in patients who are treated with autologous stem cells taken from bone marrow after G-CSF stimulation
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Background: To compare hematologic recovery between patients who received or not G-CSF stimulated (rich bone marrow - RBM)
Material and methods: The study subjects were 20 patients whose bone marrow was taken without prior stimulation of G-CSF and 15 patients in whom bone marrow was taken after the previous G-CSF mobilization. The bone marrow harvest took place on the fifth day since has been started G-CSF. Amount received bone marrow was 20 ml/kg weight.
Results: The median value of nucleated cells obtained from patients without G-CSF preparation was 3.65 x 10^8/kg. The median value of nucleated cells obtained from RBM patients was 4.83 x 10^8/kg. The median value of stem cells obtained from patients without G-CSF preparation was 0.96 x 10^6/kg body weight. The median value of stem cells derived from patients RBM was 1.9 x 10^6/kg body weight. The median time to recovery of the hematopoietic system on the basis of the increase in value PLT> 20 G/L was 12,6 days in cases of RBM and 18,8 days in cases without G-CSF preparation. The median time to recovery of the hematopoietic system based on an assessment of growth ANC> 0.5 G/L was 13,0 days in cases of RBM and 17,8 days in cases without G-CSF stimulation. It was observed statistically significant higher values of nucleated cells and increased the value of stem cells in patients with RBM compared to patients with bone marrow was taken without any stimulation (p - 0.01). It was observed faster recovery of the hematopoietic system, in cases where bone marrow was collected after G-CSF [The PLT> 20 G/L (p – 0,015) and ANC> 0.5 G/L (p – 0,01)]. Also one observed that the use of stimulated bone marrow shortens hospital stay after the administration of hematopoietic cells to 17,3 days compared with 23,1 days in patients receiving hematopoietic cells from non-stimulated bone marrow. Number of complications during transplantation in both cases was comparable. The most frequent complication was febrile neutropenia and less mucositis grade III and IV.
Conclusion and clinical importance: It seems that a better method of obtaining stem cells from bone marrow is the RBM. Using of stimulated bone marrow can faster engraftment comparing to non-stimulated bone marrow and can help patients, who fail to collect adequate number of stem cells from their peripheral blood.

P1104
Factors affecting engraftment time in patients undergoing allogeneic peripheral blood stem cell transplantation
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Objective: Engraftment delay is one of the complications seen after allogeneic hematopoietic stem cell transplantation (HSCT) that may cause mortality, morbidity and decrease the patients'

quality of life. In this retrospective study, we aimed to analyse transplantation outcomes of the patients who underwent allogeneic HSCT and to evaluate the factors affecting engraftment time.
Material and methods: The study includes 226 patients who underwent allogeneic HSCT in Erciyes Capodoccia Transplantation Center, between January 1998-January 2010. Patients' and donors' characteristics and data of apheresis products were retrospectively evaluated.
Results: 95 (42.2%) of the subjects were female, 131 (57.8%) of the subjets were male and the mean age was 29.38±10.7 years. 107 (47%) of the patients were acute myeloid leukemia, 77 (34%) of the patients were acute lymphoblastic leukemia, 22 (9.7%) of the patients were aplastic anemia and the rest were chronic myeloid leukemia, multiple myeloma, paroxysmal nocturnal hemoglobinuria. 205 (%90.7) of the patients had HLA full matched, 15 (%6.6) of them had HLA-one mismatched and 2 (%0.88) of them had HLA-two mismatched and 4 (%1.76) of them had unrelated donors. 151 (66%) of the transplanted patients did not have blood group incompatibility, 25 (11%) the patients had minor blood group incompatibility, 40 (17%) of them had major blood group incompatibility and 10 (4%) of the patients had mixed type blood group incompatibility. Peripheral blood stem cell was used in all patients. Median leukocyte engraftment time was 13 days and median platalet engraftment time was 11 days. In stem cell mobilization lenograstim and filgrastim were used. White blood cell count, mononuclear cell (MNC) count and hemoglobin concentration of donors did not have an effect on leukocyte and thrombocyte engraftment (P>0.05).
Conclusion: In this study we found that filgrastimin was superior to lenograstime when used to reach the leukocyte count 1500/µl and thrombocyte count 100000/µl in stem cell mobilizations procedure (P=0.045 and P=0.004 respectively). There was not a significant relationship between engraftment time and CD34 positive cell count, MNC count, donor leukocyte cell count, donor hemoglobin levels, apheresis time, product amount, blood group difference.

P1105
Safe and efficient PBSC collections in the smallest of children ≤ 8 Kg
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The large scale or multiple collections in the smallest of children in paediatric oncology are limited because of the potential problems of metabolic dysfunction and hemodynamic disturbance due to a high amount of pt's blood volume "catch out" in the disposable apheretic set. Moreover, the small size of the patient's veins can lead to venous access and flow rate difficulties.
Patients: From 1991 to 2010, 20 leukaphereses were performed in 11 children with solid tumors (10pts) and leukaemia (1pt). Median body weight was 7.5 kg (6.2–8kg), median blood volume was 0.56L (0.47-0.59L).
Harvest: Collections were performed daily in paediatric environment using Cobe Spectra separator. For all procedure the volume of the disposable apheretic set was more than 40% of the total pt's blood volume. Therefore for 68% of procedures the extracorporal line was primed with 150 ml of RBC, for others RBC transfusion was performed before collection. ACD was used as an anti-coagulant (ratio 1/14). Calcium was given 12 and 1 hour before and every 60 mn during collection. In 70% of procedures were realized with central lines inserted especially for the collection. In remaining 30% previously inserted (for chemotherapy) Broviac KT was used together with a peripheral vein. Flow rate were gradually increased to the maximum rate achievable and ranged from 7.7 to 16.7 mL/mn (median 10 mL/mn).
Results: A median of 2 (1-3) leukapheresis per pt were performed with a median of 2.4 (1.1-4.1) blood volumes processed/leukapheresis procedure and a median collection time 120mn/procedure. A median total $12.9x10^6$ CD34+cells/kg ($0.05\text{-}36.7x10^6$) were harvested, and 72% of children have ≥ 2x106CD34+ cells/kg with one leukapheresis. The median CD34+cells collection efficiency was 46%. Fifteen haematopoietic stem cell supports were performed. The median time to reach ANC>0.5 109/L and platelets>20 109/L was 12 and 9 days respectively.
Conclusion: Our experience shown that the problem associated with PBSC collection in the smallest children can be effectively overcome. Facilitating factors are a strong existing interprofessional team, consistent paediatric nurses involvement and enthusiastic physicians.
As PBSC collection in the smallest children are much more complicated and risky than in older children or adults interprofessional trening program which help professionals gain the skills and knowledge they need to work effectively in collection team is crucial to provide efficient and safe leukaphereses.

P1106
GCSF reduces mutated (CD27+) B-cells but increases unmutated naïve (CD27-) B-cells in stem cell donors
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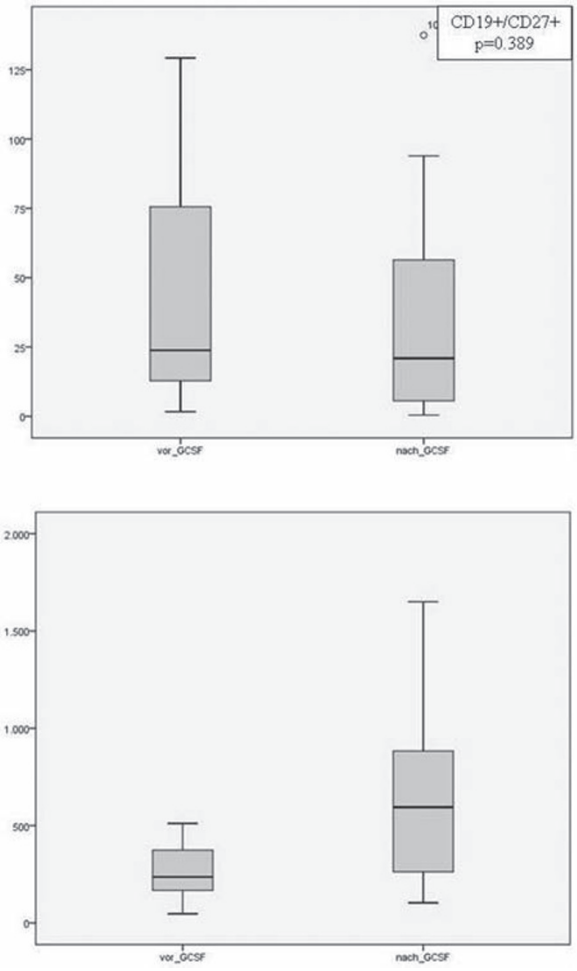
Objectives: Granulocyte colony-stimulating factor (GCSF) is commonly used for stem cell mobilization in the context of autologous or allogeneic stem cell transplantation. A number of studies elucidating the effect of GSCF on diverse leukocyte subtypes of the donor have been published so far. Little is known,



in contrast, of the effect of GCSF on unmutated naïve (CD27-) B cells and mutated (CD27+) B cells in stem cell donors.
Methods: We prospectively collected peripheral blood samples from 20 stem cell donors which were stimulated with GCSF in order to harvest stem cells for allogeneic stem cell transplantation. The first blood sample was obtained prior to the first administration of GCSF (day – 5, "before GCSF"), the second blood sample was obtained immediately prior to stem cell harvest (day 0, "after GCSF"). GCSF was administered with the common dosage of 8 applications of 7.5-8.0 µg/kg body weight. The measurement of unmutated naïve (CD27-) B cells and mutated (CD27+) B cells was performed with a five colour immune phenotype de-vice using specific antibodies for CD19, CD27, IgM, IgD and IgG.
Results: Median counts of mutated B cells (CD27+) are decreased after administration of GCSF (before GCSF: 23.8/µl ± 37.2/µl SEM, after GCSF: 20.9 ± 37.2 SEM), this reduction, however does not reach statistical significance: p=0.389. In contrast, median counts of unmu-tated naïve (CD27-) B cells are significantly increased after administration of GCSF (before GCSF: 236.0 ± 130.3 SEM, after GCSF: 594.6 ± 438.0 SEM, p<0.000).
Conclusion: To the best of our knowledge this study is the first one showing that GCSF sig-nificantly increases unmutated naïve (CD27-) B cells whereas mutated B cells (CD27+) are slightly reduced. Given that GCSF exerts effects rather on the early B progenitor cells, the increase of unmutated naïve (CD27-) B cells and decrease of mutated B cells is consistent.

P1107

High-dose chemotherapy with autologous peripheral stem cell transplantation improves remission rates in patients with relapsed acute leukaemia

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Introduction and aim: The role of APSCT(peripheral blood stem cell transplantation) in first remission patients remains controversial. Sufficient patients who relapse after chemotherapy can be salvaged with APSCT in second remission such that the beneficial effect on overall survival is blunted. APSCT produces equivalent results to ABMT but with reduced morbidity. In patients with relapsed acute leukemia, the remission rates with second line chemotherapy regimens varies from 30% to 40% percent.In this study, high dose chemotherapy followed by autolog peripheral stem cell transplantation (APSCT) were performed to provide data about contribution of APSCT to remission rates.
Materials and method: A total of 16 relapsed AML patients (with relapsed acute myelogenous leukemia) were treated with high dose FLAG regimen or EMA. The peripheral blood stem cells of the patients whom were collected after high dose ARA-C regimens in a complete remission (CR). All Patients were given PBSCs followed by HighDose Chemotherapy (FLAG or EMA) regimen. Remission rates and PFS, FFS were detected and compared after Treatment.PBSCs were infused intravenously to the patients and all results were recorded.
Results: A total of 16 patients were included in the study. 8 of the patients were male (50%) and 8 were female (50%). The median age was found as 33 (22-51) years. Infused CD 34+ stem cell count was 4,6 x 10 6/Kg (1.1–16.10 6/Kg). After the chemotherapy regimens, complete remission (CR) was achieved in 12 of 16 patients (75%) and one patient had (6%) partial remission (PR). In 2 of the patients (12%), there was no response to the treatment. No mortality was found due to high dose chemotherapy.
In 6 patients whom was infused APSC after EMA regimen, all of the patients achieved CR (100%). On the other hand; in 10 patients whom had FLAG regimen 7 patients had CR (70%) and one patient had (10%) PR. In 2 of the patients (20%), no remission was achieved. One of these resistant patients had not adequate amount of APSC (approximately 4,6 x 10^6/Kg (1.1–16.10^6/Kg). Alllogeneic stem cell transplantation (allo SCT) from HLA-identical donors was performed in 3 patients whom had CR.
Conclusion: In patients with relapsed acute leukemia, that high dose chemotherapy followed by APSCT may improve the remission rates.
Key words: Autologous peripheral stem cell transplantation (APSCT), acute myelogenous leukemia (AML), remission rate.

P1108

Preliminary experience on autologous stem cell mobilization with biosimilar G-CSF

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Biosimilar granulocyte-colony stimulating factor (G-CSF) has been proposed as a less expensive alternative to the originator product, despite missing evidence of equivalent efficacy and safety in comparative studies. In September 2009, our Hospital chose to purchase biosimilar (Ratiograstim®) instead of the originator G-CSF. Since then, 28 patients (male 17, female 11, median age 47 yrs, range 19-67) underwent autologous stem cell (SC) mobilization after receiving chemotherapy (CT) and biosimilar G-CSF. The diagnoses were myeloma/lymphoma (MM/ML) in 13 cases, acute leukemia (AL) in 12 and autoimmune disease (AD) in 3. Mobilizing regimens were intermediate dose CTX in MM/ML and AD, and high dose Ara-C in AL. Biosimilar G-CSF was administered at the dosage of 10 mcg/kg up to the last apheresis day. Apheresis was started when CD34+ cells exceeded 20/mcL. Optimal target was the collection of 6x10e6/kg CD34+ cells, with a minimal target of 3x10e6/kg. Six patients (4 AL) failed mobilization (21.4%), one of whom due to an early relapse. In the remaining 22, median time from the end of CT to the first apheresis was 11 days (range 8-17); the median number of aphereses needed was 2 (range 1-4). The median number of harvested CD34+ cells was 10.7x10e6/kg (range 2.8-40), with only 2 patients failing to achieve 6x10e6/kg. In the prior series of 28 patients undergoing autologous SC mobilization after CT and 10 mcg/kg originator G-CSF, median time to the first apheresis had been 11.5 days, with a median number of two aphereses needed. These preliminary data show that biosimilar G-CSF may be effective in mobilizing and harvesting autologous SC. Time to collection, number of aphereses and collected CD34+ cells do not differ from the corresponding figures of patients receiving the original molecule. Some concern may be raised about the rate of failures, since we had previously reported only 5 (4.9%) among 102 consecutive ML patients undergoing mobilization (Leuk & Lymph 2002). However, this heterogeneous series of patients receiving biosimilar G-CSF included a large proportion of AL (at higher risk of both harvesting failure and early relapse), and ML patients with a median age significantly higher than the previous one. Anyway, the lack of controlled studies aimed to demonstrate equivalent efficacy and safety between biosimilar and originator G-CSF is remarkable, especially considering that a positive response would undoubtedly allow the saving of significant resources.

P1109

Adverse events related to PBPC collection and mobilization for autologous transplantation in 10 years' experience: procedures, efficiency, variables related to collection and safety profile

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Objectives: We tried to evaluate the efficiency, safety and risk factors of aphaeresis procedures used for autologous PBPC collections in a 10-year period in our transplant center. Thrombocytopenia, hypotension and citrate related adverse effects were evaluated as different biological variables.
Material and methods: A total of 155 patients with hematological malignancies were analyzed (57 AML in first remission, 33 HD, 37 MM, 20 NHL, 4ALL) that underwent mobilization of PBPC. The patients were mobilized either with CTX 3gr/m² + G-CSF 10mcg/kg starting or VP-16 (2gr/m²)+G-CSF 10mcg/kg. Collections of PBSC were performed using Cobe spectra Baxter CS3000 aphaeresis system. Target of collection was >2, 0x10(6)/kg CD34+. The procedure was initiated when leukocyte count reached to 5x10(9)/L.
Results: Both regimens were effective in the progenitor cell mobilization and almost 84% of analyzed patients reached at least 2x10(6)/kg CD34+ cells with median 3 (ranges 1-6) aphaeresis procedures. In 6% of patients adequate cell dose was not reachable and overall failure rate of mobilization of 17, 5%. Furthermore 15.6% failed to harvest the optimal 4x10(6)/kgCD34+cells with >1 aphaeresis attempt. 48% patients in the CT/G-CSF group initiated aphaeresis on day 9, 34% on day 8 and 31% on day 10. Good mobilizers (GM) experienced at least one adverse event during aphaeresis compared with the no-GM. The percentage of absolute CD34+ before aphaeresis correlated with CD34+/cells/kg collected (R2=0, 62). The median of blood volume processed for body weight and the median time of aphaeresis was 7215ml (980ml-13450ml) in 202 min for GM and 8054ml (1450ml-14659ml) and 207min or no-GM. No correlation was found between CD34+/kg and volume processed. High correlation was found between the number of CD34+/kg and volume processed in the GM subject that reached the target of CD43+cells/kg only with one aphaeresis procedure (R2=0,87)
We can conclude that the mobilizing regimens were adequate to achieve PBSC harvest in 84% of pts in our center that underwent autologous transplantation. The optimal approach to remobilization strategy remains unclear. Also we did not observe any significant difference between GM and no-GM subjects in the adverse effect manifestation in reaching the CD34+cells/kg target, concerning the number of cells and volume processing. Maybe volume reducing of aphaeresis technique in future will shorten the time of achieving CD34+ target in GM subject.

P1110

Severe neurotoxicity following peripheral blood stem cell transplantation

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Introduction: Dimethyl sulphoxide (DMSO) is used as a cryoprotectant for long-term storage of hematopoietic stem cells (HSC). Various complications during infusion of cryopreserved HSC have been described. Until recently, there has been little information on the neurological toxicity of DMSO. DMSO-related neurotoxicity even coma, has been described, mostly as anecdotic events.
Case reports: We are reporting 3 cases of transient neurotoxicity out of 286 autologous peripheral blood stem cell transplantation (PBSCT) performed in our center between 2000.-2010. All patients (pts) - 2 males, 1 female, 51, 59 and 39 years old, have stage III of multiple myeloma. Each one has received Dexamethasone–based induction. A PBSC collection was performed with Cyclophosphamide (4g/m²) and rhG-CSF (5 µg/kg/day) and optimal numbers of mononuclear cells (MNC- median $8x10^8$/kgBW) were harvested. The cells were cryopreserved on 10% DMSO using a controlled–rate freezer and stored at -900C. The conditioning regimen in autologous setting was consisted of Melphalan at a dose 200 mg/m². The bags were thawed in a 37ºC water bath and infused at a rate of 10ml/min. Despite standard premedication on day "0", during infusion all pts were developed a complete loss of consciousness accompanied by incontinence, without clonic convulsions or focal neurological sings. Pulse rate and blood pressure were normal. The pts were transferred to intensive care unit (ICU) for ventilation. No laboratory abnormalities, including electrolytes, osmolarity, coagulation screen, serum glucose and enzymes, were evidenced upon repeated testing. The urgent CT scans were unremarkable. Pts were treated with steroids and forced hydration. All pts recovered consciousness in 3-5h after assistance ventilation was started. Finally, they were extubated within 24h and discharged from ICU on day +1. All pts have optimal engraftment.
Discussion: Because of the strict temporal relationship between the infusion and development of neurological sings and their resolution upon forced hydration, we circumstantially attribute the encephalopathy to the infusion of DMSO-contained in the PBSC suspension. The risk-factors for development of DMSO–neurotoxicity are still unclear. The preconditioning exposure to central nervous system (CNS)-penetrating agents and M protein in multiple myeloma pts might contribute to the occurrence of DMSO –associated neurological toxicity , but a large analyzes are needed.

P1111

In vivo plus ex vivo purging during autologous peripheral blood stem cell transplantation in high-grade B cell non-Hodgkin's lymphoma

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Background: The importance of the residual disease and the tumour contamination of the graft on relapse in lymphoma patients who underwent autologous peripheral blood stem cell transplantation (auto PBSCT) has been shown.
In this study we evaluated two commonly used B-cell purging methods, in vivo purging with rituximab and ex vivo purging with CD 34 (+) selection (CliniMacs) during auto PBSCT in high-grade relapsed B-cell non-Hodgkin's lymphoma (NHL).
Patients: Nine patients were enrolled in this study. Patients 37-78 years of age with a chemosensitive relapsed high-grade B-cell NHL (6 diffuse large cell, 3 mantle cell). Six of them had bone marrow involvement before R-DHAP regimen. The conditioning regimens were TBI+Cy (n=6) or BEAM (n=3). Five patients received rituximab after transplantation. The median CD34 (+) cells/kg infused was 1.17 x 106.
Results: No patient experienced primary or secondary graft failure. We observed no CMV and other serious infections during six months after transplantation. Median follow up was 18.1 months. Five patients are still alive and disease free. Three patient relapsed and two patients died due to progressive disease and chronic C type hepatitis. The disease free and overall survival at two years were 76% and 62.5%, respectively.
Conclusion: In conclusion, in vivo plus ex vivo purging during auto PBSCT in high-grade relapsed B-cell NHL is feasible and safe. It may play an effective role on residual disease and tumor contamination of the graft and improve outcome.

P1112

Differences in number of CD34+ cells collecting by two types of apheresis procedures and influences on kinetics of the haematopoiesis recovery

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Introduction: High-dose chemotherapy together with autologuous PBSCT is a manageable and safe therapeutic modality in treating various hemathological malignancies.
Aims of the study: The aims of the study were to obtain the adequate volume of processed blood (large volume aphaeresis vs standard apheresis)in order to achieve maximal harvest and better haematopoetic stem cell viability.
Materials and methods: The study included the patients with haematological malignancies (multiple myeloma, acute non-lymphoblastic and lymphoblastic leukaemia, Hodgkin and non-Hodgkin`s lymphoma; n=41) who underwent autologous PBSCT. Prior to transplantation procedure, an adequate mobilizing protocol was carried out depending on the type of the underlying disease together with administering a granulocytic growth factor (Neupogen®) in the dose of 5–16 µg/kg throughout a certain period of time. When leukocyte count increased, HSC harvesting procedure on cell separator began (Gambro–BCT Spectra,version 7.0). Cell resuspension in phosphate purified saline solution was analysed by flow cytometer-EPICS XL-MCL (Coulter, USA).
Results: We divided patients according to apheresis procedures. Large volume apheresis was done in 27 patients (group 1), while conventional two-days collection was conducted in 14 patients (group 2). There were significant differences in the number of CD34+ cells between of groups patients (p=0.038). Amount of infused CD34+ cells was more than $5x10^6$/kg BM in all patients of group 1. In the majority of patients in group 2 (85,7%) more than $5x10^6$/kg BM CD34+ cells can be collected in two apheresis collections. Neutrophyl engraftment (>500 /µl) occurred in median 11,26 (group 1) vs 11,7 (group 2) day (p=0,495), and platelet engraftment (>20000/µl) occurred in median 11,2 (group 1) vs 18,3 (group 2) (p=0,048). By factors analysis, we concluded that shorter period of granulocytic growth factor administration and shorter time from mobilisation chemotherapy to apheresis collecting contributed to greater harvest of HSC. Connection between CD 34+ harvest,regardless to the method of harvesting and kinetics of the haematopoesis recovery was found.
Conclusion: This is a preliminary study and number of collection is small, but overall data suggest that large volume apheresis has better efficiency for collecting more than 5x106/kg BM number of CD34+ cells in comparison with conventional two days apheresis. There were differences on platelets engraftment between both apheresis procedures.

P1113

Plerixafor in myeloma and lymphoma patients undergoing autologous stem cell transplantation

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Background: Poor peripheral blood stem cell mobilization has been reported as an obstacle to autologous stem cell transplant (ASCT). Plerixafor, a novel antagonist of CXCR4, has been recently approved as a mobilization agent for PBSC in the setting of ASCT.
Methods: We retrospectively analyzed the data on patients (“poor mobilizers”) who received plerixafor combined with G-CSF as second or third line mobilizing regimen in our institution over one-year period (n=8) and compared them to the control group (randomly picked 8 patients –with multiple myeloma and lymphoma who underwent standard mobilizing regimen using chemotherapy and G-CSF).
Results: Three out of 8 (43%) patients in the study group collected ≥$2x10^6$/kg of CD34+ cells (range: 2.02-3.71x10^6), 7/8 had ASCT, but in 4 of them the transplant material consisted also of the frozen stem cell product acquired during standard mobilization. We found that the proportion of CD34+ cells among total nucleated cells was less in the plerixafor group compared to the control group. Also the volume of frozen stem cell product was higher in the plerixafor group compared to the control group. The length of hospital stay, number of serious infections, use of antibiotics, number of blood transfusions, time to granulocyte engraftment (ANC>0.5G/l) and to platelet engraftment (PLT>20G/l) were not different between the two groups. We did not observe any side effects when using plerixafor.
Conclusions: Seven out of 8 (87.5%) of poor mobilizers were able to undergo ASCT by using plerixafor and G-CSF as a second or third line of mobilization regimen. Patients mobilized with plerixafor had similar post transplant course. Further studies regarding higher number of patients are needed to establish the above findings.

P1114

Haematopoietic stem cell mobilization with plerixafor: a single-centre experience

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Autologous stem cell transplant (ASCT) is an established treatment for many hematological malignancies. Advances in stem cell mobilization have included the development of new drugs that improve the harvest of CD34+ stem cells. Plerixafor is a CXCR4 inhibitor that enhances the “mobilizing effects” of G-CSF and thus represents an alternative for patients who failed in a previous mobilization strategy.
We hereby present the data from 8 patients who received Plerixafor in our centre. Diagnoses were: multiple myeloma (3 patients), non-Hodgkin lymphoma (4) and Hodgkin lymphoma (1). Fifty percent of the patients had failed to achieve the minimum CD34+ count required in our centre for proceeding with an autologous transplant ($2x10^6$ CD34+ cells/Kg) in a previous standard mobilization attempt; anticipating a poor harvest, the other half received Plerixafor in a first mobilization try. All patients received G-CSF (300 mcg/12h s.c.) for 5 days and plerixafor (240 ug/Kg) 11 hours before the beginning of the aphaeresis procedure. Before starting the aphaeresis and after receiving the first dose of Plerixafor, the median number of CD34+ cells in peripheral blood was 30.75/uL (range, 4.7-92.8). Therefore, the harvest could be started in all patients. The median number of aphaeresis was 2 (1-3) and 75% of the patients achieved the minimum CD34+ count required in our centre. The median number of CD34+ cells collected was $3.44x10^6$/Kg (range, 0.75-7). No adverse effects potentially related with plerixafor were observed. Eighty percent of the patients had successfully undergone an autologous stem cell transplant to date, that is, with a timely, complete and sustained graft.
In our experience, Plerixafor is a safe and effective drug for stem cell mobilization in patients who failed a previous mobilization attempt and in those who were predicted to be poor mobilizers, allowing them to proceed with ASCT.

P1115

Clinical risk management in peripheral blood haematopoietic stem cell apheresis process

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Objectives: The Hospital’s Hemapheresis unit is involved in the management of patient during post-chemotherapy phase and in Peripheral Blood Stem Cells (PBSC) collection, manipulation

and stocking.We applied FMECA (Failure Mode Effect Analysis) as clinical risk management proactive technique for errors identification and reporting.We describe the data of our project.
Methods: A multidisciplinary Team consisted of medical doctors, a hemapheresis nurse specialist and a medical laboratory technician was established. Stem cell collection process was divided into major areas: activities, owners, potential risk and error impact. On the base of adapted Scoring guidelines of VA National Centre for patient safety, we estimated frequency (scale 1-5), severity (scale 1-10) and detectability (scale 1-5) score of effects.We calculated the index priority risk (IPR) of failure modes (Delphi method).The Risk log (Master List) of possible adverse events occurrence was applied for effects with IPR>50.
Results: The FMECA team identified six consecutive steps for the hemapheresis process: Patient enrolment, Cytopenic phase/PBSC mobilization, PBSC apheresis, PBSC minimal manipulation, Cryopreserved stem cell stocking, Thawing and infusion of PBSC units.The process was separated into 27 operative areas.We calculated IPR for each area. The risk log was applied to 12 areas.Quality tools: non conformities registration of the whole process; Quality indicators: the central venous catheter (CVC) management data and the stem cell unit microbiological evaluation: three years data analysis showed that the outcomes expected values were reached in all registration; Check lists:two lists, one for monitoring each action of minimal PBSC manipulation and one to obtain a target for process education in case of enrolment of new member into the staff.
Discussion: We applied FMECA as proactive method for identify risks to patient safety and medical errors at the same time.Our work culminated in recommendations of risk reduction strategies. We implemented changes:definition of quality activity indicators, registration of data about event during more critical phases of the process, education of the staff, standardization of cryopreservation, tailored data base, tailored management software for criobiologic area.

P1116
Single-centre experience: filgrastim and lenograstim
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In this study, the effectiveness of filgrastim and lenograstim, after transplant patient, after collecting the needs of replacement donors to evaluate the relationship between G-CSF. All donors were informed about the G-CSF and apheresis procedure are taken from written consent. In the study, between January 1, 2006 and files can be accessed 16 June 2009 37 (20 male, 17 female) healthy donors were investigated retrospectively. GC-CSF to 59.5% considering donors filgrastim,% 40.5 lenograstim was used. Jugular catheter fitted to both groups. 63.7% of patients with acute leukemia in the arm donors filgrastim, lymphoma 18.2%, 18.2% other (MM, myelofibrosis and PNH) group. 73.3% of patients in the lenograstim arm of the donors acute leukemia, 13.3% AA and 13.3% of the group are lymphoma. 11.5 Mean duration of graft neutrophil and platelet graft period was 12.3 days. Average of 35 units of platelet apheresis in patients after transplantation, 3 units of red blood replacement was received. In this group, 8 females, 7 were male and the average 63 kg (44-90) and median 25 (17-49) years old. The average number of cells collected from the CD 34 +: $6.7x10^6$/kg (3.5-11.1), MNH: $6.7x10^8$/kg (2.2-12.9), volume 1022 cc, were collected and 400 min, 20 l were studied. The average entry apheresis WBC: 47.6 $x10^3$ / ml (18.9-71), Hb: 14.2 g/dl (12-16.5), Plt: $171x10^3$ ul (98-377) is. This is a retrospective study of filgrastim or lenograstim donors, CD34, MNH, engrafmen times, the red blood cell and platelet counts replaced patients, relapse and survival status, GVHD rates, the amount of product, platelet and hemoglobin levels did not differ between the 2 groups ($p \geq 0.05$). However, ionized calcium value of the processing time increases, although the difference between the two groups more than lenograstim arm numbness and contraction of the two groups in terms of defining the amount of Ca was replaced due to a statistically significant relationship ($p \leq 0.05$).

Stem cell donor

P1117
The presence of allele G in the donor for the polymorphism A7488G of the IL-17 gene improves relapse rate and survival after HLA-identical related stem cell transplantation
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Introduction: IL-17F, a member of the proinflamatory IL-17 cytokine family, has been associated with the pathogenesis of multiple autoimmune diseases. Allele G for the polymorphism A7488G, which causes a His-to-Arg substitution at amino acid 161, plays a protective role for the development of such diseases. However, its impact on the outcome of allogeneic stem cell transplantation (allo-SCT) is unknown. OBJECTIVE: To analyze the influence of the donor (D) and recipient (R) genotype for the polymorphism A7488G in the IL-17F gene on the outcome of HLA-identical related allo-SCT.
Material and methods: The study comprised 203 allo-SCT (406 D/R samples) included in the Spanish Group for Hematopoietic Stem cell Transplantation (GETH) DNA bank. The polymorphism was analyzed by RFLP-PCR (Seiderer et al. Inflamm Bowel Disease 14, 2008) and confirmed by direct DNA sequencing. Results were analyzed using the Pearson's Chi-square Test.
Results: Genotypes for D and R as well as D/R genotype combinations are shown in Table 1. No association between D or R genotype and GVHD (acute or chronic) or other complications was observed. The genotype of the donor influenced transplant outcome in terms of relapse (51/185 (27.5%) when the donor was AA vs 2/18 (11.1%) when the donor was AG; p=0.129) and survival (exitus 82/185 (44.3%) when the donor was AA vs 5/18 (27.7%) when the donor was AG; p=0,176). This was especially true for AG genotype patients (no significant differences in AA genotype patients). In fact, relapse (5/7 (71.42%) when the D was AA vs.1/15 (6.66%) when the D was AG; p=0.001) and survival (exitus 7/7 (100%) when the D was AA vs 3/15 (20%) when the D was AG; p<0.001) showed large differences depending on the genotype of the donor in this subgroup of patients. Kaplan-Meier estimates (Figure 1) showed a trend to a better event free survival (EFS; not reached (NR) vs 292 days p=0.126)) and overall survival (OS; NR vs 652 days p=0.429) in patients transplanted with AG genotype D and a statistically significant better EFS (NR vs 81 days p=0.005) and OS (NR vs 145 days p=0.022) when AG genotype patients were transplanted with AG genotype D.
Conclusions: The presence of allele G for the polymorphism A7488G of the IL-17F gene in the D was associated with lower relapse rate and better survival after HLA-identical allo-SCT. Genotyping for this polymorphism could aid in donor selection or drive a risk-adapted management of transplanted patients.

	DAA	DAG	DGG	
RAA	178 (87.7%)	3 (1.5%)	0 (0%)	181 (89.2%)
RAG	7 (3.4%)	15 (7.4%)	0 (0%)	22 (10.8%)
RGG	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	185 (91.1%)	18 (8.9%)	0 (0%)	203 (100%)

Table 1. Genotypes and D/R genotype combinations for the polymorphism A7488G in the IL-17F gene for the 203 HLA-id allo-SCT included in the study. Allele G was observed in 40/406 (9.85%) individuals corresponding to 25/203 (12.3%) allo-SCT.

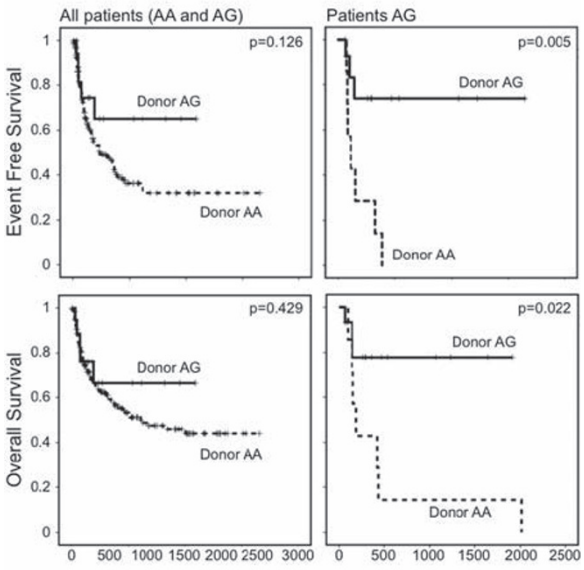

Figure 1. Kaplan-Meyer curves for event free survival (upper panels) and overall survival (lower panels) in all patients (left panels) or AG patients (right panels) transplanted from AG vs AA donors.

P1118

Unmanipulated bone marrow transplantation from haplo-identical related donor for patients with high-risk haematological malignancies

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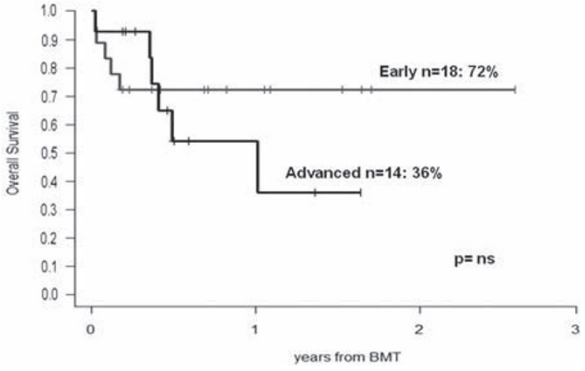
In this study we investigated the feasibility and clinical value of non T-cell depleted bone marrow transplantation (BMT) from HLA haploidentical related donor in patients with high risk hematological malignancies. Between August 2005 and May 2010, 71 patients were transplanted for AML (n=42), ALL (n=13), CML (n=5), Hodgkin lymphoma (n=5), plasmacell leukemia (n=3), myelofibrosis (n=2) and myelodisplastic syndrome (n=1). Their median age was 35 years (5-71). Thirty nine patients were in early stage (CR 1, n=27; CR2, n=12) and 32 in advanced stage (CR3, n=7; active disease, n=25). All donors were HLA identical at 1 haplotype and mismatched for 2 (n=24) or 3 (n=47) loci on the unshared haplotype. Ten patients received a reduced intensity conditioning (RIC) consisting of Fludarabine (Flu) alone (n=1), Flu + Thiotepa (Thio) + Melphalan (n=2) or Thio + i.v. Busulfan (Bu) + Flu (TBF-RIC, n=7), and 61 patients received a myeloablative conditioning (MAC) consisting of Aracytin + Cyclophosphamide combined with TBI (n=7) or Treosulphan (n=11) or oral Bu (n=11), whereas the last 32 consecutive patients underwent transplant after conditioning with TBF-MAC. All patients received an identical graft-versus-host disease (GvHD) prophylaxis consisting of Fresenius Antithymocyte Globulin combined with Cyclosporine, Methotrexate, Mycophenolate Mofetil and Basiliximab. Marrow cells were harvested from all donors after priming with Filgrastim at 3-4 microg/Kg/d from day -7 to -1 and were infused unmanipulated on day 0. The median dose of total nucleated, CD34+ and CD3+ cells infused was 7.8 (1-28) x108/kg, 2.1 (0.8-11) x106/Kg and 28 (10-98) x106/Kg, respectively. One patient had a primary graft failure. Results in terms of cumulative incidence (CI) of PMN engraftment, acute and chronic GvHD, relapse, transplant related mortality (TRM) and Kaplan Meyer overall survival (OS) are given in the Table. The 1 year OS for the 32 patients transplanted with TBF-MAC was 72% in 18 patients transplanted in early stage and 36% in 14 patients transplanted in advanced stage (see Figure). The OS for 10 patients who received a reduced intensity conditioning was 66% at 1 year. These results show that BMT from haploidentical donor using unmanipulated marrow cells and an intensive regimen for GVHD prophylaxis is correlated with high engraftment rate, low incidence of acute and chronic GVHD, reasonable TRM and favourable patient outcome.

CI of PMN engraftment at day 30	93±0.1
CI of acute GvHD grade II - IV	24±0.3
CI of acute GvHD grade III - IV	4±0.06
CI of limited chronic GvHD	8±0.3
CI of extensive chronic GvHD	5±0.1
CI of relapse at 2 years	29±0.8
CI of TRM at 6 months	28±0.3
CI of TRM at 2 years	33±0.4
OS at 3 years (early stage, n=39)	54±9
OS at 3 years (advanced stage, n=32)	26±10

Results are given as % ± standard error



P1119

CD34+ cell content in unrelated allogeneic peripheral blood stem cell grafts transported internationally. Are collection and transplantation centers' results comparable?

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The CD34+ dose represents an important graft quality indicator which affects the engraftment kinetics and patient survival. The significant clinical importance of CD34+ enumeration requires low intra- and interlaboratory variability to provide accurate engraftment potential assessment and to allow the comparison of the transplant results among centers. The aim of the study was to reveal the comparability of the graft evaluation performed and reported in different transplant centers.

Methods: We performed retrospective analysis of the results of unrelated PBSC grafts evaluation. All grafts were measured in our collection and transplantation centre and at the same time in foreign partner centers. The CD34+ percentage and absolute count (x 108) were compared. The methodological details of CD34+ enumeration were not assessed. The study was conducted for two time periods: 2003 – 2004 and 2007 – 2010. The impact of the unique laboratory number (ULN) was analyzed for both periods together.

Results: Spearman analysis showed very strong correlation of CD34+ % and CD34+ x 108 among centers for both observed periods (see Table 1.). Wilcoxon test didn't show any statistically significant differences between results obtained in compared centers (ns). Variability of all measurements ranged from 10,6 to 13,8 percent. The quality of correlation was dependent on the laboratory identity. The correlation coefficient of CD34+ % wasn't statistically significant for only one laboratory (r=0,410, ns). Case CD34+ x 108 was also found to be statistically insignificant for three laboratories (r=0,200; 0,285; 0,624, ns).
Conclusion: The excellent correlation of CD34+ content measured on the same grafts by independent transplant and collection centers confirms the low interlaboratory variability and high level of standardization of CD34+ enumeration. The results of CD34+ cell doses reported by different transplant centers in various studies can probably be relied on and one can expect the same results again. The study documents and underlines the importance of activities keeping the low interlaboratory variability, such as external quality control schemes, interlaboratory measurements, workshops etc.

Table 1.

	our centre		partner centers		Spearman correlation r	p
2003 – 2004 (n =56)						
CD34+ % (median)	0,54	0,18-1,54	0,46	0,10-2,06	0,899	<0,0001
CD34+ x 10^8 (median)	3,83	0,31-12,21	3,84	0,42-13,50	0,966	<0,0001
2007 – 2010 (n = 98)						
CD34+ % (median)	0,69	0,20-1,68	0,70	0,13-1,77	0,922	<0,0001
CD34+ x 10^8 (median)	5,29	0,84-13,45	5,42	0,61-13,23	0,880	<0,0001

P1120
Poor mobilization in allogeneic peripheral blood stem cell donors - predictive parameters and consequences
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Objectives: CD 34+ cell dose is a crucial issue in peripheral blood stem cell (PBSC) transplantation. We present an analysis of factors associated with poor PBSC mobilization in healthy donors and report the follow up data.
Methods: 6901 healthy related and unrelated donors who donated PBSC`s between 1/1996 and 9/2010 were prospectively documented in a database. According to the yield of CD 34+ cells in the 1st apheresis the donors were divided into 3 groups:
Group 1: < 2x10^6/kg recipient weight (n=159; 2.3%)
Group 2: > 2x10^6/kg < 3x10^6/kg recipient weight (n=282; 4.1%)
Group 3: > 3x106/kg recipient weight (n=6460; 93.6%)
The groups were analyzed regarding to age, sex, BMI, baseline leukocyte and platelet count and frequency of 2nd donation of G-CSF mobilized PBSC. The follow up assessments of blood counts at 1 month, 6 months, and annually up to 5 years after PBSC donation were evaluated. Data shown represent median values and ranges, Chi-square- Test and ANOVA were applied.
Results: Table 1 shows characteristics of the donors in the 3 groups. Poor PBSC yield in the 1st leukapheresis was clearly associated with female sex, higher age and lower BMI of the donors. Leukocyte and platelet counts at baseline did not show remarkable differences among the 3 groups. The percentage of donors undergoing 2nd PBSC donation within 2 years were 14 (8.8%) in group 1, 15 (5.3%) in group 2 and 152 (2.4%) in group 3. During the follow up period, leukocyte and neutrophil counts were significantly lower in group 1 with very poor PBSC yield.
Conclusion: A poor CD 34+ yield of an allogeneic PBSC donation is associated with donor characteristics (age, sex, BMI). Donors with poor mobilization results are at higher risk for a 2nd donation request. Poor mobilizing donors should be monitored with special accuracy during the follow up period.

Donor characteristics	Group 1 < 2x10^6/kg	Group 2 > 2x10^6/kg < 3x10^6/kg	Group 3 > 3x10^6/kg
Age (years)	**39.0** (1 – 74)	**34.0** (19-73)	**33.0** (5-75)
Sex male/female (%)	**50/109** (31.4%/68.6%)	**120/162** (42.6%/57.4%)	**4702/1758** (72.8%/27.2%)
Body Mass Index	**22.58** (16.14-35.75)	**23.04** (17.43-36.49)	**24.90** (13.43-57.53)
Baseline leukocyte count (x 10^9/L)	6.01 (2.99-16.54)	5.95 (3.00-14.25)	6.24 (2.29-19.02)
Baseline platelet count (x 10^9/L)	240 (66-391)	234 (115-461)	246 (99-579)

Table 1: Donor characteristics in the 3 groups according to the CD 34+ yield from the 1st leukapheresis

P1121
HLA-A/B/C/DRB1/DQB1 haplotypes contributing to successful donor search in unrelated haematopoietic stem cell transplantation
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Objective: At the time of search initiation, the prediction of donor search outcome is of great value for the physicians to delineate the strategy of patient care. The duration and success rate of an unrelated donor search greatly depends on the expression in patients of at least one of the well-known 10 most frequent Caucasian HLA haplotypes. Unfortunately, few (10-20%) Caucasian patients express such haplotypes. Here, we aimed at finding additional common haplotypes to improve the prediction of successful search.
Method: Typing results from 774 families of pediatric patients waiting for a hematopoietic stem-cell transplantation were initially examined. The selection criterions for subsequent inclusion were HLA-haplotype assignment with certainty by family segregation analysis and clear documentation of Geographic origin of families. HLA broad HLA-A/B/DRB1 haplotypes that were expressed with frequencies ≥ 0.19% in patient families of European origin and that split into ≤ 2 predominant four-digit HLA-A/B/C/DRB1/DQB1 haplotypes were refereed as common. Expression of at least one of those in 168 patients of various geographic areas with no family donor was subsequently relied to the chance of finding ≥ 9/10 HLA-matched unrelated donors.
Results: 50 common four-digit haplotypes were identified. A higher ($P<5.10^{-6}$) chance of finding a suitable donor was found for 53/168 (32%) recipients that expressed at least one of these common haplotypes. Considering these common haplotypes, the patients were divided into 2 probability groups. The high probability group included patients that express i) at least one of the 50 four-digit common haplotypes and no more than 2 rare alleles or rare B/C or DR/DQ associations on the other haplotype, or ii) no common haplotype and no more than one rare allele or unusual association on both haplotypes. The remaining patients and also those with ≤ 3 suitable donors on BMDW registries were classified into a low probability group. The rates of successful search were 97% and 26% in the two groups respectively.
Conclusion: Prediction of search outcome could be improved by including these 50 common haplotypes into the current estimate process.

P1122

The Potential Application of the Allogeneic Transplant by the Policy of Widespread Donor Search: an Intention-To-Treat Analysis from the Rome Transplant Network

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The policy of the Rome Transplant Network (RTN) for patients candidated to an allogeneic hematopoietic stem cell transplant (HSCT) and lacking a HLA identical sibling is the contemporary search for an alternative HSC source such as Matched Unrelated Donor (MUD), Cord Blood (CB) or Haploidentical Related Donor (HRD). The main aim of the RTN policy is the identification of a suitable donor in order to perform transplant in adequate timing. The selection criteria for MUD consist of a 8/8 HLA loci matching tested at low resolution for class I HLA and at high resolution (HR) for class II. Selection criteria for single CB unit are based on cell doses (TNC>3x10^7/kg and CD34+>1x 105/kg) and on a > 4/6 HLA antigen compatibility. From April 2006, the haplodentical option was also simultaneously considered, so all closer family members have been tested for the HLA. Here, we report the results of the intention to treat (ITT) analysis on the potential therapeutic impact of our transplant policy. Data were obtained from RTN database. From April 2006 to June 2010, 379 (92%) out of 413 candidated pts have been considered eligible to receive an allogeneic HSCT for hematological disease. HLA identical sibling donor was available in 135 out of 379 (36%) cases, while a search process for an alternative donor was activated for 244 (64%) pts. Eight (3%) are too early to be evaluated and we were not able to identify any alternative donor for 21 (9%) of the 236 remaining pts. An alternative donor was identified for 215 (91%) of these 236 pts. Despite the identification of an alternative donor, 43 (20 %) pts lost the eligibility during the search process because of several causes. To date, 172 (80%) of 215 pts have been definitively transplanted (67 MUD; 43 CB; 48 HRD) or are willing to proceed towards the transplant (n=14), while 135 pts have been grafted from a HLA identical siblings. In summary, a suitable donor was identified for 350 (93%) of 379 pts eligible for an allogeneic transplant, which could be performed in 307 (81%) of them. From this ITT analysis, we can conclude that, by adopting the RTN policy of widespread donor search and multiple transplant options, the allogeneic transplant can be offered as potential therapeutic procedure to a large majority of pts.

P1123

One-week family donor follow-up after peripheral blood stem cell collection: results of a donor questionnaire in a prospective study

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Healthy donor follow-up is an important part of assessing the incidence of short- and long-term side effects and adverse events after peripheral blood stem cell (PBSC) collection. Although stem cell mobilization and collection is generally well tolerated, information on donor health in the first period after donation is often obtained through questionnaires sent to donors some time afterwards when the exact memory of the critical first few days has faded.
In 1996 we started a prospective short- and long-term family PBSC donor follow-up project including blood cell count evaluation at 7 days, 4-8-12 months after donation and annually thereafter. From January 1996 to June 2010, 283 PBSC family donors (132 men, 151 women, mean age 40 years) performed 435 apheresis procedures. On completing their collection cycle, donors were given a questionnaire on which to report possible symptoms during the following week. The questionnaire asked about symptom occurrence, duration and perceived intensity (graded as mild, moderate or severe), the need for drug assumption and the length of time off work.
157 (55%) questionnaires were returned 7 to 15 days after donation; 23 donors reported no symptoms, 40 mild symptoms, 85 moderate symptoms and 9 severe. Moderate/severe symptoms most frequently meant: fatigue (n=62), bone-muscle pain (n=51), headache (n=25), malaise (n=21), dizziness (n=10); the median symptom duration was 5 days (range 1-15). Among donors who reported moderate/severe symptoms 23% needed drug assumption and 41% reported a median time of 5 days off work (1-10). Among less frequent events: 2 cases of skin rash; 2 of gout, 1 of orchitis. Though not a perceived symptom, it should be noted that 3 donors had a transient thrombocytopenia (platelet count between 71-87 x 10^9/L) 7-10 days after donation, despite being normal when the procedure ended.
Conclusion: no serious adverse events were reported by the healthy PBSC donors who returned the one-week follow-up questionnaire, though 60% of them suffered from moderate to severe symptoms lasting for 5 to 15 days in 57% of cases; a minority of donors needed the help of drugs and prolonged resting time. The incidence of less frequent events may have been underestimated. Strict donor follow-up in the first few weeks after donation could contribute to better assessment of the incidence of side effects, giving donors more realistic information.

P1124

Incidence and impact of G6PD deficiency in haemopoietic stem cell donors: a retrospective analysis in a Sardinian centre

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymatic disorder of red blood cells in humans. The G6PD enzyme catalyzes the first step in the pentose phosphate pathway, leading to antioxidants effect protecting cells from oxidative damage. A G6PD-deficient patient lacks the ability to protect red blood cells from oxidative stresses from drugs, metabolic conditions, infections and ingestion of fava beans. Because drug-induced haemolysis is considered the most common adverse clinical consequence of G6PD deficiency confusion exists regarding which drugs can cause haemolytic anaemia in these subjects. In absence of general consensus patients are subjected to conflicting advice for medications they can assume and for medical procedures they can be submitted (i.e. general anaesthesia). For all these reasons and lack of exhaustive data in the literature, G6PD deficiency has been considered a risk factor for BM and PBSCs donation. In our centre G6PD levels are routinely screened in all potential donors therefore we could evaluate incidence and consequences of this deficiency. Since January 2002 to October 2010, 96 donors were evaluated; mean age was 43 years (range13-70). Forty-nine (49) were females, 47 males. Seventeen (17) donors presented G6PD deficiency (17.7%).G6PD deficiency was complete (mean value 0.07 mU; range 0.01-0.3) in 10 and intermediate (mean value 0.66 mU; range 0,41-0,94) in 7 donors. Thirteen (13) donors performed PBSCs donation and 4 bone marrow donation in general anaesthesia. In the normal donor population 49 gave PBSCs and 30 bone marrow. There were no differences between the two groups of donors and all followed the same protocol for medications and supportive care. After the donations there were 3 mild adverse events among the normal donors (3.7%); 1 fever, 1 allergic reaction, 1 respiratory insufficiency. All were in BM donors and all recovered in less than 48 hours. No adverse event was observed in G6PD deficient donors. No significant haemolysis was observed in the donors in the early and late follow up. Patients' red cell engraftment was regular. All patients converted to donor G6PD status after transplant. Our experience indicates that an healthy G6PD-deficient subject can safely

donate both bone marrow and peripheral blood stem cells. This is an important information most of all in those regions in which G6PD deficiency is diffuse and for a possible different approach in the volunteers donor selection.

P1125

Access to allogeneic haematopoietic stem cell transplantation in the Netherlands for paediatric patients with MDS and AML in first relapse treated according to protocols of the Dutch Childhood Oncology Group

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Allogeneic hematopoietic stem cell transplantation (aHSCT) is a curative therapy for malignant diseases of childhood. However, it is suspected that not all patients in need of an aHSCT are offered one. Apart from donor availability and the deterioration of the patients' clinical condition during the unrelated donor (UD) search process, the literature identifies other barriers for access to an aHSCT. Most studies focus on patients for whom a donor is identified or who already have been transplanted. As a consequence, a significant number of patients eligible for an aHSCT might have been excluded. Children with malignancies are registered at diagnosis at the Dutch Childhood Oncology Group and those who are diagnosed with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) in first relapse are considered eligible for aHSCT. Having accurate disease incidence data and protocol indicated eligibility for aHSCT provides a unique opportunity to investigate access to aHSCT. If not all eligible children have access we also aim to identify possible reasons and solutions. Children (<18 years) diagnosed with primary MDS (n=90) or AML in first relapse (n=75) between 1998 and 2008 were included in this retrospective study. Six patients were not eligible for protocol treatment and were excluded. For 48 (30%) patients a family donor was identified, for 90 (57%) patients an UD search was performed and for 21 (13%) patients no UD search was initiated. These 21 patients were divided into patients with and without access to aHSCT according to the recorded reason not to perform an UD search. Reasons include: no (second) complete remission (n=10), conserve quality of life (n=1), stable disease (n=3), immunosuppressive therapy (n=2), patient died (n=3), patient lives abroad (n=1) and second relapse (n=1). Looking at the time interval between date of diagnosis and date of death/last follow up, for seven (4.4%) patients it may be questioned why an UD search was not performed. Poor clinical condition may have caused omitting the UD search; however looking at the time intervals these seven patients should have had access to aHSCT, there seems to have been enough time to initiate an UD search. In conclusion, the fact that most children are given the option of an allogeneic HSCT is encouraging and reasons not to transplant seem legitimate in most cases. Whether this holds true for other malignancies or adult patients remains unknown and may be subject of future studies.

P1126

Allogeneic haematopoietic stem cell transplantation with reduced-intensity conditioning in patients with high-risk multiple myeloma. Comparative analysis of outcomes between unrelated and related donor

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The purpose of this study was to assess the results of allogeneic stem cell transplantation (Allo-SCT) after reduced-intensity conditioning (RIC) from an unrelated donor in patients with high-risk multiple myeloma (MM) in a single centre. From January 2007 to January 2010 we transplanted 33 consecutive patients with MM. Thirteen (39%) (Group 1) and 20 patients (61%) (Group 2) had unrelated and related donor respectively. The median age was 48 years (39–63) in the first group and 56 years (40–67) in the second group.

Thirty two patients (97%) received one or more autologous transplantation. Twenty five patients (Group 1: N=4 (31%); Group 2: N=4 (20%) were treated with a RIC based on Fludarabine (30 mg/m²/d x 5); Busulfan (4 mg/kg/d p.o. or 3.2 mg/kg/d IV over 2 to 3 days) and rabbit ATG (2.5 mg/kg/d x 2). Seven patients received Fludarabine (25 mg/kg/d for 3 days) and 2 gray TBI and 1 patient Fludarabine (40 mg/m² x 3 days), Cyclophosphamide (60 mg/kg/d x 2) and 2 Gy total body irradiation (TBI).

The median follow-up was 17 months (4-39). None of our patient experienced a graft rejection. The cumulative incidence of grade II-III acute graft versus-host disease (GVHD) was higher (38%) for the unrelated donor vs (15%) for the related donor (p=0.12). The cumulative incidence of chronic GVHD was no different between the first and second group (31% vs 30% respectively). At last follow up 27 patients (group 1: N=12 (92%); Group 2: N=15 (75%)) were still alive of whom 15 are in CR (group 1: N=7 (58%); Group 2: N=8 (53%)), 9 in PR (group 1: N=4 (31%); Group 2: N=5 (33%) and 3 in progressive disease (group 1: N=1; Group 2: N=2). The estimated probability of non relapse mortality (NRM) at day 100 was 0% in the two groups. At two-year the NRM probabilities was lower in the unrelated group 14% vs 24% in the related group (p=0,477). Also at 2 years, patients receiving unrelated transplantation had superior overall and progression-free survivals, 83% and 65% respectively compared to patients with related donor transplantation, 67% and 36% (p= 0.241). The incidence of acute GVHD, OS, PFS and NRM were not significantly different between the two groups.

Conclusion: In patients with high risk multiple myeloma; RIC Allo-SCT with unrelated donor, is feasible and effective treatment with low non-relapse mortality, high complete remission rates and prolonged disease-free survival. This procedure seems to be comparable to those of HLA-identical siblings.

[P1125]

Table 1. No UD search

	n (%)	Survival interval[1] (days) median (range)	Alive n
Valid reason			
No CR[2]	5 (3.1)	40 (7-63)	0
Conserve quality of life[3]	1 (0.6)	1330	0
Stable disease[4]	3 (1.9)	1115 (404-1547)	3
Immunosuppressive therapy[4]	2 (1.3)	266 (202-329)	2
Died before aHSCT	3 (1.9)	23 (5-41)	0
Subtotal	*14 (8.8)*		*5*
Questionable reason; no access to aHSCT			
Patient lives abroad	1 (0.6)	2331	1
No second CR[5]	5 (3.1)	122 (80-123)	0
Second relapse[5]	1 (0.6)	92	0
Subtotal	*7 (4.4)*		*1*
Total	**21 (13.2)**		**6**

[1]Time interval between date of diagnosis and date of death or last follow up; [2]MDS patients; [3]Patient also had tetraplegia; [4]MDS refractory anemia (RA) or refractory cytopenia (RC) patients; [5]Relapsed AML patients; UD: unrelated donor; CR: complete remission; aHSCT: allogeneic hematopoietic stem cell transplantation.

P1127

Descriptive and comparative analysis of the Portuguese Bone Marrow Donor Registry

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The present work is the result of the analysis of 155677 donors in terms of the distribution of HLA-A, -B, -DR antigens and haplotypes in all Portuguese districts, including the islands of Madeira and Azores. Expressing the differences between districts as fixation indices, or FST, by using an Analysis of Molecular Variance (FST representing genetic distance between each pair of districts), we also created a neighbour-joining phylogenetic tree, in an attempt to identify closely related populations within the country. The highest frequency HLA-A antigens are: 02, 01, 03, 24 and 11. HLA-A02, 01, 03 and 24 are the four most frequent in all districts, but A01, 03 and 24 vary in their relative position. HLA-B44 is the predominant antigen in all districts with the exception of Portalegre, where B35 is more common; B35 and B51 are the second and third most common antigens in most districts. HLA-B51 is not the third most frequent antigen in Bragança and in the islands of Madeira and Azores (replaced by B14 in Madeira and B07 in Azores). The predominant HLA-DR antigens are DR07 in nine districts and DR13 in eleven districts. In the DR07 districts, DR13 comes second in eight of them; in six of these eight districts, including Lisboa and Porto, DR04 is the third DR antigen. The five HLA -A, -B, -DR haplotypes more frequently encountered were 01-08-03, 29-44-07, 02-44-04, 33-14-01 e 03-07-15. Only one more haplotype, 02-44-07, has a frequency higher than 1%. The six and the twenty five most common haplotypes in the country account for, respectively, 9.904% and 23.3716% of the entire registry. Among the districts, the differences observed concerned the relative frequencies of the predominant haplotypes, with Madeira, Beja, Castelo Branco, Bragança and Viana do Castelo being the ones with the most irregular distributions. Some haplotypes that differed among the regions were 02-51-08, 02-51-11 and 02-07-15. In the estimation of the neighbour-joining phylogenetic tree, a distribution of the districts can be observed in an almost geographically accurate position. The islands of Madeira and Azores appear more distant genetically. Our results suggest that the Portuguese Registry should focus in trying to recruit new donors in Madeira and Porto Santo, Viana do Castelo, Castelo Branco, Bragança and Évora.

The authors wish to express their gratitude to the CEDACE HLA typing laboratories, CHSul, Centro de Histocompatibilidade do Norte and Centro de Histocompatibilidade do Centro.

P1128

MicroRNA expression profiling changes along G-CSF treatment and at long term in CD34+ cells from healthy donors

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Background: Different sources of hemopoietic progenitor cells are used in the allogeneic stem cell transplantation (allo-SCT) setting. All of them show different effects after the transplant such as time of engraftment, hemopoietic recovery and complications following allo-SCT. Furthermore, G-CSF exerts a methylating effect and alters the microRNA (miRNA) expression profiling. We hypothesized that G-CSF effect could be responsible for a long term change in the miRNA expression profiling following G-CSF administration. The aim of this study was to analyze the G-CSF effect in long term on miRNA expression profiling in CD34+ cells in healthy donors and to compare it with the miRNA profiling before G-CSF and at fifth day of G-CSF administration.
Methods: 16 samples from CD34+ cells from peripheral blood at steady state levels (PB), at fifth day (5d) and at 30th (30d) day of G-CSF administration were analyzed in healthy donors. The expression of 375 miRNAs was analyzed using TaqMan Human MicroRNA Arrays v2.0 (Applied Biosystems). miRNA expression data was analyzed by the 2^{-Ct} method. Hierarchical clustering of miRNA expression data was performed using single linkage and Euclidean distance. ANOVA and t-test were performed to identify differentially expressed miRNAs between groups. Analyses were performed with Multiple Array Viewer. Functionality of the differentially expressed miRNAs was determined from TAM database.
Results: hierarchical cluster analysis showed a general differential pattern miRNA profiling at the 30d group. Comparison among all groups showed a changing pattern of six miRNAs differentially expressed. More detailed analysis showed that after G-CSF (5d) there was an increased expression of miR21 and miR100 and a down-regulation of miR148b, miR128 and miR24; miR21 was still over-expressed at 30d, and miR24 expression increased from 5d to 30d. Further, a late effect was detected at 30d with up-regulation of miR139, miR143 and miR145 which were at higher levels respect to PB and 5d groups. miR21, miR143, miR145 and miR148b are involved in human embryonic stem cell regulation. miR21 is also involved in apoptosis, cell cycle, granulopoiesis and immune response; miR143 is involved in hormone regulation and miR145 in cell proliferation, cell cycle and acts as a tumour suppressor gene.
In summary, the relevant function of these miRNAs overexpressed at 30th day after G-CSF administration suggests a miRNA regulation at long term due to G-CSF administration.

P1129

Faster registration on international donor registries and shorter time to allogeneic HSCT after having found a donor confers better outcome in acute leukaemia patients

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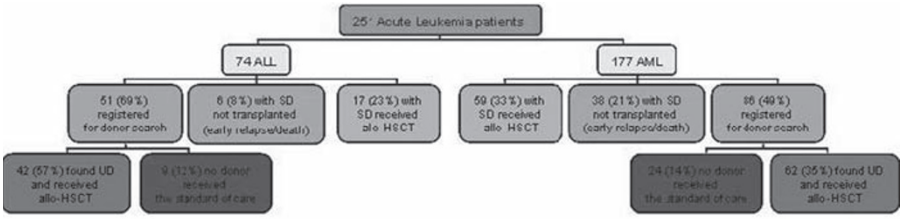
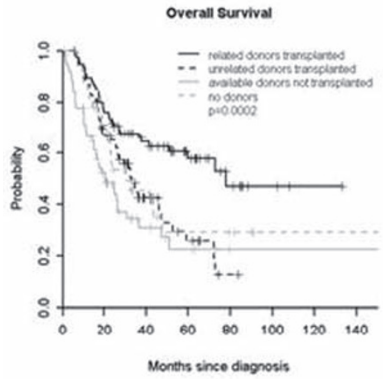
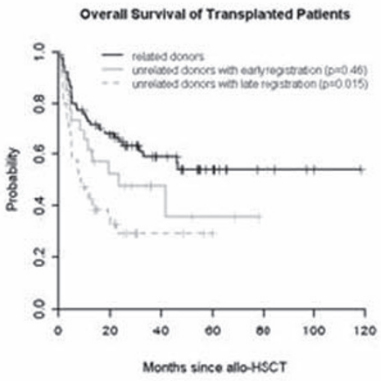
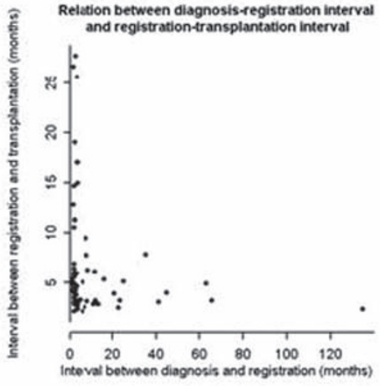
A patient has 30% chance to find an HLA identical sibling donor (SD) and approximately 40% chance to find a suitable unrelated donor (UD), 40% of registered patients relapse or die before finding a donor. We evaluated the outcome in acute leukemia (AL) patients, whether they had an HLA identical SD, an UD or no donor (ND) after registration on France Greffe de Moelle (FGM) registry, either transplanted later or not. Secondary objectives were to evaluate the impact of intervals diagnosis-allo-HSCT, donor finding-allo-HSCT, registration-allo-HSCT, on OS and EFS. We analyzed 251 AL patients, 117 (47%) males and 134 females, median age at diagnosis 40 years [16-66], 177 (71%) AML and 75 ALL. Seventy six (30%) patients had an available SD and received allo-HSCT within a median time of 3.5 months (0.5–43) and 38 (15%) had SD but were not transplanted due to early relapse and/or death. For patients with no available SD, a registration on FGM registry was done, 137 patients were registered after a median interval of 2.3 months (0.4-135) from diagnosis, 33 (13%) patients did not find any donor and they received the standard of care; 104 (41%) patients found an UD or UCB unit after a median time of 1.6 months (0.3-26): 86 with UD of which only 60 have been transplanted within a median time of 2.3 months (0.4–14), 18 with UCB of which only 17 were transplanted. Among transplanted patients, 113 (74%) were in CR, 40 in <CR. Fifty (33%) received PBSC, 86 (57%) received BM and 17 (10%) UCB units. For conditioning, 56 (37%) were RIC and 96 standard. For HLA, there were 45 HLA 10/10, 14 HLA 9/10, 1 HLA 8/10 and for UCB 14 HLA 4/6 and 3 HLA 5/6. After a median follow-up of 25 months (0.2-234), the median OS was 78 months (51–133) for transplanted patients with SD (3years OS: 68%), it was 33 months (27–47)

[P1129]









for transplanted patients with UD (3years OS: 44%), 21 months (15–37) for not transplanted patients with available SD or UD (3years OS: 34%) and it was 31 months (23-221) for patients with ND (3years OS: 45%). Median EFS for the same groups was 38 months (23–133), 24 months (17–36), 15 months (11-24) and 23 months (14–48) respectively. In multivariate analysis, 3 significant factors affected OS: disease status (<CR) HR=2.8 [1.5-5.3] p<0.001; long interval diagnosis-registration HR=2 [1.2-3.6] p=0.001 and conditioning (standard) HR=0.27 [0.1-0.8] p=0.02. The interval diagnosis-registration appeared as major factor affecting survival in UD allo-HSCT settings.

P1130

Favourable outcome of single locus related mismatch HSCT for class I antigen in patients of Eastern Mediterranean background

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Introduction: The Eastern Mediterranean (EM) region consists of communities that are ethnically and genetically homogenous1. The objective of this study is to evaluate the outcome of our adolescent and adult patients treated with allogeneic HSCT from single locus related mismatch for Class I antigen compared with HLA full match related donors.

Methods: We retrospectively reviewed our adult patients who had an allogeneic HSCT from single locus related mismatch for Class I antigen during the period 1996- 2010 (study group). A manual matched analysis at a ratio of 1:1 was done to compare this group with a cohort of patients who received an HLA full match related donor HSCT (control group) during the same period of time. Both cohorts were matched for diagnosis, disease status, age, sex, conditioning and GVHD prophylaxis (Tab 1) .

Results: A total of 19 patients were identified with a median follow up of 20 months (study group) and 29 months for the control group (P=0.4). There was no statistically significant difference in the outcomes (OS, DFS, cumulative incidence of relapse (CIR), incidence of aGVHD and cGVHD) betweens the 2 groups (Tab 2).

In univariate analysis, outcomes for the study group were comparable at 3 years, with an OS of 71.1%, DFS of 65.7% and CIR of 34.7% compared with 79%, 64.7% and 30.4% respectively for control group (hazard ratio [HR]OS =1.4; 95% CI 0.4-5.4; P = 0.6, HRDFS = 0.99; 95% CI 0.3-3; P=0.98 and HRCIR =1.01; 95% CI 0.3 -2.9; P= 0.9).

Also, the 3-years cumulative incidence of acute and chronic GVHD for the study group were comparable with those for the control group (26.6% and 39.5% vs 27.7% and 38.7% respectively) with HRaGVHD =0.8; 95% CI 0.5-2.2; (P= 0.7) and HRcGVHD = 0.7; 95% CI 0.2-2.9; (P= 0.5).
Conclusion: The outcome of single locus related mismatch HSCT for Class I antigen is comparable to the outcome of HLA full match related donors in our ethnically and genetically homogenous patient population. Larger studies with the same set up are needed to validate this result with other centers in the EMRO or other regions.
Reference:
1. Aljurf MD, Zaidi SZ, El Solh H, Hussain F, Ghavamzadeh A, Mahmoud HK et al. Special issues related to hematopoietic SCT in the Eastern Mediterranean region and the first regional activity report. Bone marrow transplant (2009) 43, 1-12.

Table 1. Patients' Characteristics

	Matched analysis	
	Study group (n=19)	Control group (n=19)
Follow up, months		
Median (range)	20	29
Age, years		
median (range)	18.6(14 -50)	19(15-49)
Sex (No %)		
Male (No %)	14 (73.7%)	15(78.9%)
Female (No %)	5 (26.3%)	4(21.1%)
Year at transplant		
median (range)	2006(1996 -2010)	2004(1997-2010)
Diagnoses (No %)		
ALL	10(52.6%)	10(52.6%)
AML	5(26.3%)	5(26.3%)
CML	1(5.3%)	1(5.3%)
SAA	1(5.3%)	1(5.3%)
NHL	1(5.3%)	1(5.3%)
Thalassemia	1(5.3%)	1(5.3%)
Disease status at Transplant (No %)		
CR1	12(63.3%)	12(63.3%)
CR2	3(15.8%)	3(15.8%)
CP1	1(5.3%)	1(5.3%)
PIF	2(10.5%)	1(5.3%)
SD	1(5.3%)	2(10.5%)
Conditioning (No %)		
BU/CY	4(21.1%)	7(36.8%)
BU/CY/ATG	2(10.5%)	0
CY/TBI	11(57.9%)	11(57.9%)
FLU/CY	1(5.3%)	1(5.3%)
BU/FLU	1(5.3%)	0
GVHD prophylaxis (No %)		
CSA/MTX	15 (78.9%)	17 (89.5%)
CSA/ATG	1(5.3%)	0
CSA/MTX/ST	2(10.5%)	1(5.3%)
CSA/MTX/ST/CY	1(5.3%)	1(5.3%)
Stem cell source (No %)		
BM	17(89.5%)	16(84.2%)
PB	2(10.5%)	3(15.8%)

Table 2. Outcomes

Outcomes	Probability (95 % CI)				
	Study group	Control group	P*	HR (95% CI)	P**
aGVHD(grade II-IV)	26.6%(26.5-26.7)	27.7%(27.6-27.7)	0.9	0.8 (0.5-2.2)	0.7
cGVHD	39.5%(39.4-39.5)	38.7%(38.6-38.7)	0.8	0.7(0.2-2.9)	0.5
CIR	34.7%(34.6-34.7)	30.4%(30.3-30.4)	0.5	1.01(0.3-2.9)	0.9
DFS	65.7%(65.4-65.9)	64.7%(64.1-64.5)	0.98	0.99(0.3-3)	0.98
OS	71.1%(70.7-71.2)	79%(78.7 – 79.3)	0.6	1.4 (0.4-5.4)	0.6

* Logrank test for OS & DFS and Fine & Gray for others
** HR

P1131
Allogeneic stem cell transplantation from one-antigen HLA-mismatched unrelated donor increases acute II-IV GvVHD incidence without interfering with other post-transplant complications
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Only one third of patients how need allogeneic stem cell transplantation (allo-SCT) for malignancy have an HLA-identical sibling donor. We have reported that in patients with standard risk malignancy, transplantation from unrelated HLA-matched donors led to outcomes similar to those from HLA-identical sibling donors (Yakoub-Agha et al, JCO 2006). Stem cell grafts from unrelated donors are being increasingly used but more than 30% of patients are still lacking a well-matched donor. To investigate the impact of unrelated one-antigen HLA-mismatched graft, we report a single center retrospective study on 209 patients who underwent allo-CST from sibling donor (n=123), unrelated HLA-matched donor (n=73) and unrelated one-antigen-HLA-mismatched donor (9/10) (n=13) over the last 5 years. Underlying diseases were AML (n=104), ALL (n=54), myelodysplastic syndrome (n=30), and myeloproliferative syndrome (n=21). Of the 117 males patients, 23% received graft from female donor. Medians age of recipients and donors at transplantation were 45.2 years and 40.8 years, respectively. Patients received conditioning regimens using either myeloablative (n=149): 81 who received High-dose TBI (12Gy) or nonmyeloablative (n=60): 48 who received low-dose TBI (2Gy). Antithymoglobulin was given to 25 pts. Bone marrow was the main source of stem cells (72%). Results: with the median of follow-up of 37.9 months, 78 patients died including 25 from TRM. Relapse was recorded in 70 patients. Seventy-two patients experienced acute GVHD (aGVHD) including 43 with II-IV grades and 26 with III-IV grades. In multivariate analyses, donor type and conditioning were the most important risk factors negatively influencing the overall survival (p=.002; HR=2.038 and p=.016; HR=1.81, respectively) and event-free survival (p=.005; HR=1.783 and p=.015; HR=1.728, respectively). Conditioning type influenced the risk of relapse (p=.048; HR=1.699) while donor type was found to influence TRM (p=.030; HR=2.428). Graft from unrelated one-antigen HLA-mismatched donor was the foremost risk factor for aGVHD grade II-IV (p=.019; HR=2.663; [95%CI: 1.178-6.019]). In conclusion, except for aGHVD II-IV, allo-CST from unrelated one-antigen HLA-mismatched donor, seemed to led to outcomes similar to those from HLA-identical unrelated donor and may be considered as an alternative option for patients without a full-matched donor. Prospective studies are warranted, however, to confirm our data in larger cohort of patients.

P1132
Presence of immunoreactive minor histocompatibility antigens disparities in HLA-matched siblings and syngeneic twins
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Objectives: Disparities of minor Histocompatibility antigens (mHA) have been considered as an important immunogenetic factor influencing immune responses following transplantation from unrelated donors. The aim of our study was to answer whether immunogenic mHA incompatibility in either Host-versus-Graft or Graft-versus-Host direction is present and thus may influence the outcome of allo-HCT from HLA-matched siblings and syngeneic twins.
Methods: We have analyzed 23 patients (pts) with AML transplanted from HLA (A,B,C,DRB1,DQB1) matched siblings and 3 pts transplanted from syngeneic twins in the Dept. of Hematology and BMT, Katowice, Poland. Preparative regimen was Bu+Cy or Treosulfan+Fludarabine. Alleles encoding 11 minor Histocompatibility Antigens (mHA: HA-1, HA-2, HA-3, HA-8, HB-1, ACC-1, ACC-2, HwA-9, HwA-10, UGT2B17, HY) were analyzed for each donor-recipient pair with use of Dynal All-Set mHA typing kit and PCR-SSP method. Only immunogenic mHA mismatches were considered. Information on whether mHA mismatches might result in Host-versus-Graft or Graft-versus-Host responses were established with use of the minor Histocompatibility Knowledge Database of Leiden University Medical Center.

Results: Immunogenic mHA mismatches of HA-1, HA-2, HA-8, HB-1, HwA-9, UGT2B17 or HY were identified in 19(83%) sibling pairs. 14(61%) pairs presented mHA mismatched in HVG direction, 8(35%) in GVH direction. Bidirectional mHA disparity was detected in 3(13%) pairs. 4(17%) pairs presented no mHA immunogenicity. Differences in genes encoding mHA were also found in 2 out of 3 syngenic pairs: different allele of EB-1 was present in one pair, and two different alleles of HwA-9 and HwA-10 were present in second pair.
Conclusion: Immunogenic mHA mismatches may be present in alloHCT recipients from HLA-matched sibling donors and thus they may be potentially responsible for occurrence of transplantation complications. Disparate mHAs evidenced in monozygotic twins question the belief that they are genetically identical. Continuation of the study is warranted to establish the associations of specific mHA mismatches with alloHCT outcomes.

P1133

Three days of G-CSF priming are as effective as 5 days of priming for obtaining bone marrow-derived stem cells from matched related allogeneic donors

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Purpose: To evaluate the efficacy of 3 days of granulocyte-colony stimulating factor(G-CSF) priming for collecting bone marrow-derived(BM) stem cells from normal pediatric donors in matched-related allogeneic transplantation,and to compare the results with published study using 5 days of G-CSF mobilization in 42 healthy pediatric donors (pediatric blood and marrow transplant consortium-PBMTC-study, Blood.2007;110:4584-4587).
Patients and methods: Retrospective chart review of all pediatric patients who received matched-related allogeneic bone marrow transplantation between January, 2005 and October, 2010 at King Hussein Cancer Center(KHCC), Jordan from G-CSF-primed BM from normal pediatric donors was performed.
All donors received 5 mcg/kg per day of G-CSF as a single subcutaneous injection for 3 consecutive days. BM was harvested on the third day under general anesthesia,with a target volume of 15-20 mL/kg of patient's weight.
Results: a total of 42 children were included, 19 were males and 23 females. The median age was 8.5 years (0.7-18),17 donors (40%) were between 0-6 year of age, 11 (26%) between 7-12 year, and 14 (24%) between 13-18 year. No donor experienced major adverse events related to G-CSF administration or marrow harvest. The median dose of nucleated(NC)and CD34+ cells infused per recipient weight was 5.2 $x10^8$/kg (1.5-10.2 $x10^8$) and 5 $x10^6$/kg (1.6-12$x10^6$), respectively (compared to 6.7$x10^8$/kg of NC and 7.4$x10^6$/kg CD34 cells in the PBMTC study).
The median age for the patients was 5.6 years (0.2-16). Nineteen were males and 23 females. Thirty-five patients had non-malignant diseases that include: Thalassemia (n=21), sickle cell anemia (n=1), bone marrow failure (n=7), inborn error and immunodeficiency disorders (n=6). While 7 patients had malignant disease that include: ALL (n=3), AML (n=1), CML (n=3).
All patients engrafted, with median neutrophil and platelets engraftment time of 15 days (10-22) and 22 days (13-42), respectively. The median follow up time was 41 months (4-69). The estimated overall survival and event-free survival at 5 years was 95% and 83%, respectively.
Conclusion: Collection of G-CSF primed BM form pediatric donors below 18 years of age is as safe and as efficient as that of 5-days of G-CSF priming. Infused cells facilitate neutrophil and platelets engraftment with equal efficiency as 5-day primed cells. Our results indicate that G-CSF administration can be reduced to 3-days which can minimize short and long term adverse effects of G-CSF use in healthy pediatric donors.

P1134

Peripheral blood progenitor cells collection in healthy donors for allogeneic transplantation: experience of a single center with large volume leucapheresis

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Introduction: The use of peripheral blood cells in allogeneic transplant programmes have been extended universally in the last decade due to advantages in comparison with bone marrow. However, there are no defined guidelines to improve the efficiency of collections. We present our experience using Large Volume Leucapheresis (defined as more than 3 times the total blood volume) for collection of PBPC in healthy donors, applied in our centre with the aim of simplifiing the method.
Methods and donors: 124 healthy donors (66 males and 58 females; median age 48, 19-73) were included in this study since November 2001 to December 2010. All donors received 12-14 µg/kg/day s.c of rhG-CSF (filgrastrim®, Amgen, Thousand Oaks, CA, USA) in two doses during 4 days for mobilization. Our main objective was to yield at least 3x10e6/kg receptor weigth of CD 34+ cells. Leucapheresis was started on the fifth day after the administration of rhG-CSF. Large Volume Leucapheresis (LVL) was programmed and progenitor cells were collected through peripheral vein access in most cases, using a Cobe Spectra separator (COBE SPECTRA, Gambro BCT, Lakewood, CO, USA). Intraprocess controls were performed to finish the procedure according the objectives and results.
Results: PBPC collection yield a median of 6.02x10e6/kg CD 34+ cells (2.85-13.57). The median number of patient's blood volumes processed was 3 L (1-4). 113 donors (92%) required only one session to achieved the CD34+ cells objective. No mobilization failure was observed. All products were transplanted with rapid and sustained engraftment in all cases. No serious adverse effects were observed and minor morbidity related to the PBPC collection was scarce and reversible. Asymptomatic mild thrombocytopenia was transitory and turned to normal within days. More details about the procedures results will be presented.
Comments: In our experience Large Volume Leucapheresis (LVL) facilitate the collection of blood progenitor cells in healthy donors. This method allows an adequate PBPC collection for transplantation with the simplification of a single harvesting procedure which is enough for prompt hematological engraftment.

P1135

Factors affecting donor satisfaction after matched sibling stem cell donation in Chinese, a telephone questionnaire follow-up survey

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Background: The World Marrow Donor Association (WMDA) recommends post donation follow-up for hemopoietic stem cell transplantation (HSCT) donors for potential health problems. In Hong Kong, the Hong Kong Red Cross Transfusion Service conducts follow-up for unrelated donors with the WMDA questionnaire. Sibling donor reassessment is carried out by Queen Mary Hospital HSCT center.
Material and methods: The WMDA questionnaire of 5 questions (relating to pain, health, hospitalization, medical follow-up and overall satisfaction) is administered. Donors that refuse or are lost to follow-up are excluded.
Results: Among 785 sibling donors, a total of 216 answered the questionnaire. They included 98 men and 118 women. Bone marrow was harvested in 165 cases and peripheral blood stem cell (PBSC) in 51, at a median of 6.6 years (range 0.5 to 19) ago. The median age at donation was 38.9 years (range 8 to 68)

while the current median age was 45.4 years (range 18 to 74). A total of 43 patients complained of significant pain (41 donating BM) while 69 had health complaints (51 BM donors). Hospital visits occurred in 77 cases (mostly for body checkup), while 15 cases requested further referrals. Graphically, these four features were evenly distributed in terms of years from donation. They were unrelated to current or donation age, survival of HSCT recipient or BM volume harvested. On an analogue scale of 1-10 (least to most satisfied), the mean satisfaction score was 7.45 (SD 2.02). Satisfaction was higher in PBSC donors than BM donors (7.98 vs 7.33 years), but PBSC donors were also older (45.7 vs 35.8 years) and had more recent donation (3.9 vs 7.4 years). On multivariate analysis, only recent donation showed a trend to higher satisfaction (p=0.053).
Conclusions: Most sibling donors have a positive perception of the donation experience. The most frequent negative impressive arise from pain during BM donation. More structured follow-up of HSCT donors is needed to help us to understand their problems and to recognize their contribution to the HSCT service.

P1136

Lower viable CD34 recovery in cryopreserved allogeneic PBSC compared to autologous PBSC

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Allogeneic peripheral blood stem cells (PBSC) are cryopreserved less often than autologous harvests. The use of cryopreserved allogeneic HPC is becoming increasingly common for storage of excess HPC or T cells for donor lymphocyte infusions, in addition to circumventing issues of donor availability particularly in light of recent worldwide events (Sept 11, swine flu and flight disruptions due to volcanic ash).
During 2006 to 2009, our cell processing laboratory cryopreserved 30 allogeneic and 350 autologous PBSC. It was noted that the post-thaw viable CD34+ recovery was lower in allogeneic PBSC products (median = 63%, range 16-92) than autologous (72%, 13-151; p <0.0004). Hence this study aimed to determine factors that influence post-thaw CD34 recovery.
We analysed data from all cryopreserved allogeneic and autologous PBSC, with the aim of determining the effect of cryopreserved nucleated cell concentration (NCC), neutrophil content, and time from collection to cryopreservation.
Univariate analysis demonstrated weak inverse correlations were between viable CD34 recovery and NCC (Spearman r=-0.20, p<0.0001), collection to freeze time interval (r=-0.10, p=0.048), neutrophil content (r=-0.20, p<0.0001). 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).
Of the 35 cryopreserved allogeneic products, 11 have been infused with no significant difference in terms of engraftment of neutrophils (median 18; range 11-31) and platelets (median 27; range 14-58), when compared to infusion of 179 fresh allogeneic products (neutrophils: median 17, range 4-55, p=0.9; platelets: median 19, range1-101, p=0.2).
This data indicates that the lower post-thaw viable CD34 recovery in PBSC may be due to intrinsic properties of allogeneic donor in addition to the neutrophil content and prolonged storage periods prior to cryopreservation. Post thaw analysis of viable CD34+ content is recommended to ensure sufficient viable CD34+ to facilitate engraftment.

P1137

Cytoreductive therapy with clofarabin followed by HLA-haplo-identical BMT/HSCT in advanced/refractory haematologic malignancies and in relapse after allogeneic HSCT

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14 patients were transplanted from an HLA-haploidentical donor at our center between August 2009 and October 2010. Patients (pts) characteristics: The age of the patients ranged from 24 to 70 yrs, with a median of 42.5 yrs. 11 pts were male, 3 female. 8 pts had an AML or sAML: 5 were relapsed after prior HLA identical HSCT; 2 were relapsed after initial chemotherapy and 1 pt had a progressive sAML (all three without a matching donor). 2 ALL pts: both relapsed after prior HLA identical transplantation. 2 CLL pts: 1 pt with refractory disease and 1 pt with refractory and transformed disease (both del p53). 2 Lymphoma pts: 1 refractory mantel cell lymphoma after autologous PBSCT and 1 pt with high risk T-NHL in 2nd CR.
Therapy: Cytoreduction with Clofarabin (30mg/m2/5 days) was given to 10 pts. The conditioning regime consisted of Fludarabin (30mg/m2) day -6 to-2, Cyclophosphamide (14,5mg/kgBW) day -6 and -5, TBI (2Gy) day -1 or Melphalan 110mg/m2 day -2 and HSCT (11 BM/ 3 PBSCT) on day 0. G-CSF was administered from day +1 until take and immunosuppression consisted of post transplantation high dose Cyclophosphamide (50mg/kgBW) day +3 and +4, MMF and Tacrolimus.
13 of 14 pts had a leukocyte take (range day 14 to 20), 1 pt died on day 12 without take in acute liver failure, 1 pt died on day 42 because of GvHD and infection (TRM 14%), 3 pts had an acute GvHD grade III/IV (21%), 4 pts are relapsed (2 dead, 2 alive). 9 pts are currently in complete remission and alive.
Conclusion: Alternative haploidentical donor transplantation using a pretransplant cytoreductive therapy with Clofarabin and posttransplant immunosuppression with cyclophosphamide seems to be an alternative approach for patients with high risk advanced or refractory hematologic malignancies even in relapse after HLA-identical allogeneic HSCT. Clofarabin is able to induce responses and remissions in different hematologic malignancies. Rates of TRM and incidence of grade III/IV aGvHD are acceptable so far. In the majority of patients a complete remission could be induced, the remission data are preliminary and have to be reevaluated 2 years after HSCT.

P1138

Related donor screening, management and early adverse events in peripheral blood haematopoietic stem cell collection for adult HLA-identical and haplo-identical allogeneic transplantation: an analysis of approach of different Italian apheresis centres

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Objective: Potential eligibility of related adult donors within allogeneic stem cell transplantation setting.
Method: We retrospectively evaluated full charts of 500 candidate donors (May 2005-December 2009) from 9 Apheresis

Centers affiliated to Società Italiana di Emaferesi e Manipolazione Cellulare (SIdEM) in Lombardy, Piemonte and Liguria.
Results: Overall, 6 centers applied eligibility criteria for blood donation while 5 centers applied Italian Bone Marrow Donor Registry standards; 7/9 centers were JACIE-certified, and 4 centers had no protocol for second donations. Data of potential donors from 8/9 centers (51% male, 49% female; median age 47 yrs, 13-77) showed that 71 candidates (14%) were ineligible for donation: 63 for both peripheral blood stem cell (PB) and bone marrow (BM) (5 refusals, 58 clinical problems, mainly cardiovascular); 6 for PB (autoimmunity) and 2 for BM (1 refusal, 1 clinical problem). 352 donors (53% male, 47% female; median age 45 yrs, 13-76) donated PB (508 collections), 50 donated BM. Granulocyte colony-stimulating factor (G-CSF) mobilization, in charge of transplant team in 6/9 centers, is fairly homogeneous. Apheresis was started, regardless of the day of collection, with Hemoglobin (Hb) <12.5 in females or <13.5g/dL in males in 17% of donors, platelets <150g/L in 18% (<80 g/L in 0.9%), white cells >70g/L in 2% and CD34+ <10/µL in 1%. A central venous catheter (CVC) was used in 6 centers (8% of donors). A yield of CD34+ ≥2x10^6/Kg was achieved in 97% of donors. Adverse events (AE) occurring within 7 days were mild in 23% of aphereses (paresthesia and peripheral access problems) and severe in 0,8% (collapse, severe vaso-vagal reactions, non fatal-arrhythmias). Changes in blood counts at day 7 mainly affected Hb level; nevertheless, no blood transfusions were needed and autologous platelets reinfusion was performed in 3/9 centers. No CVCs related AE were reported. G-CSF-related toxicity occurred in 93% of mobilizations without severe AE or deaths. Bone pain was observed most frequently (60%) followed by myalgia (13%), headache (7%), asthenia (6%), fever (3%), nausea (2%), abdominal pain (0.6%) and splenomegally (0.6%), arrhythmia (0.3%).
Conclusions: No clinically relevant early AE related to G-CSF, CVCs and apheresis were observed in our donor. More uniformity in donor management and a prolonged follow up applied at a national level should be planned to guarantee the safety and quality of donation in related donors.

P1139
Automated peripheral blood haematopoietic progenitor cell anaylsis on the Sysmex XE2100 and prediction of peripheral blood stem cell harvest timing
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SW Peninsula Transplant Service performs approx 120 peripheral blood stem cell harvests (SCH) a year. Optimal harvest collection time post mobilisation was previously predicted by peripheral blood white cell counts (PBWBC) and, for borderline or abnormal pattern PBWBCs, a peripheral blood CD34+ (PBCD34) cell count was used. Sysmex XE2100 analysers are implemented with the capability for automated Haematopoietic progenitor cell (HPC) enumeration as part of a routine full blood count. We looked at the utility of HPC as a quick automated method to refine the PBWBC alone as the trigger for harvesting with the aim to minimise PBCD34 flow cytometry, thus reducing laboratory costs and delays in commencing apheresis in the clinic.
We studied PBHPC counts retrospectively for 2 years. Correlations between pre-harvest PBHPC and PBWBC counts with CD34 yields were compared in 93 harvests Harvest yields at various ranges of PBHPC counts were analysed.
Of SCH producing CD34 yields of <0.8 x 10^6/kg, 45% had PBHPC counts of <10/ul and 79% had PBHPCs of <20/ul. In autologous SCH, correlation between the PBHPC count and CD34+ cell yield (r=0.71) was substantially higher than that between the PBWBC and yield (r=0.07). No autologous harvests with PBHPC counts of <10 cells/ul produced a CD34 yield >0.7 x 10^6/kg. 93% of PBHPCs >50 produced 'worthwhile' CD34 yields of >0.7 x 10^6/kg and 65% were above target yield of >2 x 10^6/kg.
In allogeneic SCH (n=15) 100% of harvests achieved yields >0.7 x 10^6/kg, including PBHPC counts of <10 cells/ul.
Investigations were performed into stability and reproducibility of this parameter showing that stability of HPC results deteriorated after 2 hours in EDTA and reproducibility was improved by using the mean count from 3 consecutive tests.
This lead to a strategy for routine use and prospective evaluation:
Autologous harvests:
Mean of 3 HPC counts performed within 1 hour:
PBHPC <10/ul – indicates no harvest
PBWBC <8 or abnormal pattern and HPC>50/ul, use PBCD34 to avoid missing optimal harvest time
PBWBC>8 but PBHPC 10-20/ul, use PBCD34 to avoid poor harvest
PBWBC>8 and HPC>20, perform harvest
Allogeneic harvests: Use of HPC not beneficial
An audit of 29 subsequent harvests showed, after one year of routine use, an improvement in timing of SCH and will be presented. Specific examples of the use of the HPC to prevent missing good yields and avoid poor harvests will be demonstrated.

P1140
The role of campath in HLA-C mismatched unrelated allogeneic transplant: a single-centre experience
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HLA class I mismatch (MM) has an important impact on the outcome of unrelated donor transplantation (URD). The role of HLA-C MM is controversial. It is associated with increased risk of graft versus host disease (GvHD), graft failure and reduced overall survival. T-depletion with Campath has been associated with a lower incidence of GvHD, but it increases graft failure and relapse in URD. We report a retrospective analysis on the outcome of 23 patients (pts) with HLA-C MM who underwent URD bone marrow transplantation (BMT) with T-depletion with Campath.
A total of 23 pts with HLA-C MM had an URD transplant with conditioning regimens which included Campath from 6/2002 and 11/2010. Pts received conditioning regimen according to local protocols and in 4 cases a reduced intensity conditioning (RIC) was used; GvHD prophylaxis consisted of calcineurin inhibitor + methotrexate for myeloablative BMT and + mycophenolate for RIC. Acute and chronic GvHD (aGvHD-cGvHD) were graded according to standard criteria. Engraftment was defined as neutrophil recovery > 1.0x109/L in 2 consecutive days. CMV, HHV6 and EBV were monitored periodically and treated as appropriate.
The male-female ratio was 8:15, with a median age of 20.2 years (4.1-58.1). Diagnosis were: 5 acute myeloid leukemias, 5 acute lymphoblastic leukemias, 4 myelodisplastic syndromes, 3 severe aplastic anemias, 2 Hodgkin lymphomas, 2 Non-Hodgkin lymphomas, 1 chronic myeloid leukemia and 1 acute byphenotypic leukemia. Most pts received peripheral blood stem cells. Sixteen pts had exclusively HLA-C MM (13 serological and 3 allelic). Twenty-one pts were evaluable for engraftment; 19 engrafted. Median time for engraftment was 19 days (12-37). Graft failure occurred in 2 pts. Seventeen pts developed aGvHD mainly grade I-II and 12 developed cGvHD, extensive in 3 cases. Viral reactivation occurred in 18 cases. Nine pts died, 4 of bacterial sepsis, 1 of viral encephalitis, and 3 of relapse. Six pts showed clinical or molecular evidence of relapse. Overall survival at 1 year was 56.5%, with a median follow-up of 625 days.
We report a single institution series of 23 pts with HLA-C MM and Campath T-cell depletion. The rate of primary graft failure

was 8.6% and no secondary graft failure was seen. Although 74% pts experienced aGvHD, none experienced grade IV GvHD. Relapse occurred in 26% pts. Although our series is small and heterogeneous, it shows that Campath may have a role in HLA-C MM allogeneic unrelated transplant.

P1141

Immunoglobulin-like receptors (KIR) assessment on bone marrow donor and host

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The natural killer (NK) cells are one kind of lymphocytes and have a special function on immunological response. There are two principal paths: missingself and missingligand, both are used to induce alloreactivity in NK cells. Aim: to evaluate the alloreactivity response of NK cells based on presence or not of KIR (immunoglobulin-like receptors) and HLA genes in bone marrow donor and host. Methods: From March 2009 to February 2010 in the Hemocentro of University of Campinas 20 pts (patients) were assessed based on NK alloreactivity. A sample of 150 µl total blood was taken by donors' and hosts' genomic DNA, using EDTA tubes and extract by EZ-DNA Kit. The PCR-SSO technique was used for genotyping HLA-A, B, C, DR, DQ, 14 KIR genes and 2 pseudogenes, besides that an agarose gel was performed to confirm the reaction. The product was hybridized with microbeads specifically designed to genes and alleles of KIR and HLA, the flow citometry technique was used to detect the beads binding on PCR product. Results: 20 pairs were analyzed of identical HLA, 17 AML (Acute Myeloid Leukemia) pts, 2 CML (Chronic Myeloid Leukemia) and one ALL (acute lymphoid leukemia). None of pairs had significant NK alloreactivity. However, 4 showed an haplotype KIR host type B and donor type A, HLA-C group 1 with the presence of ligands KIR2DL2/3, two had severe chronic Graft versus Host disease (GVHD) and 2 did not; 10 pts had haplotype host and donor B, HLA-C group 1 and 2 and the presence of ligands KIR2DL2/3 and KIR2DL1, 7 had severe chronic GVHD, 2 acute GVHD and 1 died for relapse; 2 pts showed haplotype host and donor B, HLA-C group 2 in the presence ligands of KIR2DL1 and one pt died for relapse; 2 pts presented haplotype donor and host B, HLA-C group 1 with ligands KIR2DL2 and had chronic GVHD; 2 pts had haplotype host B and donor A, HLA-C group 2 with ligands KIR2DL1 and the occurrence of severe chronic GVHD. Conclusion: More tests will be conducted to establish a correlation between KIR/HLA of donors and hosts of allogeneic bone marrow and with haploidentical donors and hosts to elucidate which influence KIR can have on the response after bone marrow transplantation.
Support: CNPq

P1142

Portuguese Bone Marrow Donors Registry (CEDACE): recipient post-transplant follow-up

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Background/Objectives: CEDACE, the Portuguese Bone Marrow Donors Registry was created in 1995, although it only started effective growth in 2003 (December 2003 figures 6133 registered donors), when an increasing donor registration slope started. In September 2010 there were 237 130 registered donors and 201 collections had taken place in the 3 Collection Centres (Porto Oncology Institute, Lisbon Oncology Institute and Lisbon Santa Maria Hospital). Concerning the development of Portuguese Bone Marrow Donors Registry, different objectives of quality and donor patient evolution control are developed. The establishment of patient evolution follow-up is considered a priority for CEDACE. In this abstract we present the preliminary results of this activity in a two year follow up.
Methods: During October 2010, CEDACE sent the "Patient update request post transplant" form to the Transplant Centres where the Bone Marrow/Peripheral Blood Stem Cell (BM/PBSC) were transplanted, asking for information about the outcome and clinical situation of the 55 (2009) and the 24 (1st semester 2010) BM/PBSC transplantation recipients. Additionally, that information would constitute feedback information to the respective unrelated Portuguese Stem Cell donors (CEDACE).
Results: 26 (47%) answers out of the 55 requests sent concerning 2009 transplanted patients were received. 18 (75%) answers out of the 24 requests concerning the 1st semester of 2010 transplanted patients were received.
Concerning patient clinical situation for 2009, 14 (54%) out of the 26 patients are alive and well. 8 (31%) were deceased. 3 had disease relapse and 1 Thrombocytopenic Thrombotic Purpura. For 2010, 12 (63%) out of the 18 patients are alive and well. 4 (21%) were deceased and 3 other patients are having/ had clinical problems such as Guillan Barré Syndrome (1 patient) septic shock (1 patient) and disease relapse (1 patient).
Conclusion: It was not possible to obtain feedback information about all transplanted patients' clinical condition and that was more important in the 2009 follow up (29 lacking out of 55 vs 6 lacking out of 24 of the 1st semester 2010). That may be related to a number of issues that CEDACE will continue to find ways to overcome.
Overall results related to the clinical situation of the respondents are encouraging: 54% of the patients transplanted in 2009 are alive and well as well as 63% of the ones transplanted in the 1st semester of 2010.

P1143

Cardiac biomarkers abnormalities in healthy donors in the course of mobilization with granulocyte-colony stimulating factor: case report and preliminary data from a single centre

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We report on a case of non-ST elevation myocardial infarction occurring in a healthy donor undergoing peripheral blood stem cell (PBSC) mobilization with granulocyte colony stimulating factor (G-CSF).
The donor was a 52 year old man, heavy smoker, with moderate hypertension under treatment. He never had cardiovascular symptoms before. ECG and exercise treadmill test were normal. Screening for thrombophilia (antithrombin III, protein C and protein S, antiphospholipid antibodies, anticardiolipin antibodies, homocysteinemia, Factor V Leiden mutation, Factor II G20210A mutation, APC-resistance) was negative. He started G-CSF 300 mcg twice daily, and after 4 doses he complained of chest discomfort. Rising values of troponin I (TnI) and Creatine kinase-MB (CK-MB) were observed after 7 doses of G-CSF, and peak value was 0.88 ng/mL and 15.9 ng/mL, respectively (laboratory upper reference limit: TnI < 0.06 ng/mL; CKMB < 3.5 ng/mL). Mobilization was interrupted and the patient was admitted to intensive Cardiac Care Unit. ECG was normal. No pathological findings were observed after echocardiography. Angiogram did not show significant coronary disease. The donor was discharged on day +7.
This finding prompted us to search cardiac biomarkers abnormalities in 5 additional consecutive donors (1 unrelated, 4 sibling; 2 male, 3 female; median age 49, range 28-67). No one experienced symptoms of myocardial damage. Mobilization

and apheresis was successfully carried out in all cases. TnI was measured at baseline before G-CSF administration and at various time points in the course of mobilization (AccuTnI, Access Immunoassay Systems, Beckman Coulter). Baseline values were normal. In all these donors higher TnI levels were observed after G-CSF, with peak values exceeding upper reference limit (median 0.15 ng/ml, range 0.06-0.24).
Conclusions: cardiac biomarkers are not routinely determined in asymptomatic donors. TnI alterations have been observed with a high frequency in our small series, in the absence of clinical evidence of myocardial ischemia. In the presence of normal baseline values, TnI and CK-MB elevations might be associated with subclinical myocardial injury and might be predictive of major adverse cardiac events. Since donor safety is a major issue in the setting of peripheral blood stem cell (PBSC) donation, the clinical impact of cardiac biomarkers elevation should be studied in a larger series and the underlying mechanism should be investigated.

P1144
Soluble CD40L levels in bone marrow donation
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Introduction: Soluble CD40 ligand (sCD40L) is a member of the tumor necrosis factor family and was shown to modulate inflammatory and thrombotic reactions. Additionally, it is well known that sCD40L levels are clearly affected by preanalytic conditions, especially by platelet activation. Therefore we examined sCD40L levels in peripheral as well as in bone marrow (BM) derived blood samples.
Methods: In five healthy BM donors, plasma (anticoagulated by EDTA (1.8 mg/mL)) of peripheral blood (PB) as well as of BM derived blood (harvested by puncturing the iliac crest) were collected and compared to the respective serum samples. Additionally, sCD40L was measured in the respective BM derived cell product (anticoagulated by citrate (5.5 mg/mL) and heparine (10 IU/mL)). sCD40L levels were determined by an commercially available ELISA-Kit (R&D Systems).
Results: In comparison to PB plasma (238 ± 137 pg/mL) sCD40L levels of BM plasma (1330 ± 593 pg/mL) and of BM derived cell products (941 ± 425 pg/mL) were significantly elevated. However, sCD40L levels of BM plasma were within the same range as BM serum samples (1529 ± 394 pg/mL). Furthermore, platelet count in BM derived cell products (117±26 x 10*9 platelets/L) was reduced compared to PB (296±42 x 10*9 platelets/L).
Conclusions: In BM plasma samples, sCD40L levels were elevated up to a range typical for serum samples indicating an insufficient anticoagulation by standard EDTA concentrations for these blood samples. In BM derived cell products showing reduced platelet counts compared to peripheral blood, sCD40L levels found to be significantly lower than the respective serum values, indicating a sufficient inhibition of platelet activation and suggesting that sCD40L concentrations in bone marrow are clearly higher than in peripheral blood.

P1145
A comparison of Auto-PBSC and MNC (Cobe Spectra). A pilot study
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Background: Peripheral blood stem cells (PBSC) are used as source for stem cell transplantation. Cobe Spectra's MNC program is the most used method for PBSC collection, but auto-PBSC is fully automated and therefore not dependent on operator interactions. Auto-PBSC has been used in our centre with a high collection efficiency (CE) in patients (median 58 %), but less satisfactory in donors (median 47 %). Therefore, MNC program was introduced to collect PBSC from healthy donors.
Methods: Whole-blood to ACD ratio 10:1. Auto-PBSC, version 6.1: Harvest volume 5 ml, chase volume 3 ml. MNC, version 6.1: Collect flow rate according to white blood cells and the percentage of mononuclear cells. All donors were adults, healthy, related or unrelated.
Results: Four donors donated one day with auto-PBSC and one day with MNC, all women, median age 51 (43-64), table 1. In 2010 a total of 18 collections in 12 donors with auto-PBSC and a total of 7 collections in 6 donors with MNC were performed, table 2.
Conclusion: In our small study the auto-PBSC and the MNC program are equally suitable for collecting PBSC from healthy donors. The numbers are small and more donors will be included. We expected the MNC to show a significant shorter collection time, which is the great benefit compared to auto-PBSC. The faster collection, the more blood volume can be processed and the bigger chance of collecting the requested yield. A whole-blood-to-ACD ratio of 12:1 would probably result in a shorter collection time.

Table 1	Median number (range)*	
	Auto-PBSC	MNC
Pre-aphaeresis CD34+ (/ul)	68 (29-109)	50 (22-97)
WBC (x 10^9/l)	53.8 (47.8-76.7)	57 (28.6-74.5)
Percentage mononuclear cells	17 (15-18)	16 (13-17)
Inlet volume (ml)	10680 (10000-11930)	10500 (8520-12710)
Inlet volume (x blood volume)	2.3 (2.3-2.5)	2 (1.6-3)
Time (min)	253 (247-263)	216 (151-275)
CD34+ collected (x 10^6)	382 (213-637)	302 (150-475)
Collection efficiency (%)	52 (49-73)	57 (39-77)
volume of product (ml)	161 (88-192)	218 (172-262)

* p > 0.05 (Wilcoxon rank sum test, two-tailed)

Table 2	Median number (range)*	
	Auto-PBSC	MNC
Age	49 (40-67)	59 (43-70)
Men/women	4/8	3/3
Pre-aphaeresis CD34+ (/ul)	66 (19-122)	39 (10-123)
WBC (x 10^9/l)	58 (28.9-82)	59 (28.6-74.5)
Percentage mononuclear cells	15 (8-18)	13 (10-17)
Inlet volume (ml)	12265 (6000-17680)	12710 (8520-18740)**
Inlet volume (x blood volume)	2.3 (0.8-3.1)	2.5 (1.6-3)
Time (min)	253 (118-313)	235 (151-275)
CD34+ collected (x 10^6)	328 (84-817)	210 (86-1037)
Collection efficiency (%)	48 (13-77)	48 (39-59)
volume of product (ml)	153 (88-296)	240 (172-289)

* p > 0.05, ** p = 0.001 (unpaired t-test, two-tailed)

P1146
First step towards Macedonian donor registry-mobilization of HLA-identical familiar healthy stem cell donor in allogeneic transplant setting
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Mobilized peripheral blood stem cells (PBSC) from healthy donors have become an increasingly used alternative to bone marrow for allogeneic transplantation. Granulocyte colony-stimulating factor (G-CSF) –primed peripheral stem cells harvesting may result in a graft with increased mononuclear cells collected, increased progenitor cell dose and potential for more rapid engraftment resulting in improved survival. Filgrastrim is not only known to mobilize CD34+ progenitor cells but acts as a pleiotropic immune modulator. So, systematic donor follow-up in healthy donors is needed.
The aim of this study is to evaluate safety and feasibility of G-CSF primed hematopoietic peripheral stem cells in familiar

HLA-identical donors. The follow-up focused on clinical and laboratory testing including reports of adverse event after the mobilization.
Granulocyte colony-stimulating factor (G-CSF) is administrated in 56 healthy donors to reach sufficient mobilization in the period 2000-2010. The donors were characterized as follows: 43 years median; female 60% of the donors. G-CSF was administrated in the dose 10µg/kg of donor weight in five day and PBSC collections started on the fifth day using COBE Spectra cell separator. The aim was to collect mononuclear cells 2x108/kg of recipient weight. Three donors were mobilized twice (for second transplant). Aphaeresis needed to reach target number of CD34+ cells were: 1 apherese in 50%, more than two apherese need in only 1 patient. The most frequent adverse event that was noted by patients was bone pain associated with increasing number of white blood cells. Better mobilization and higher PBSC yield correlated significantly with younger age. Four years after G-CSF –primed peripheral stem cells harvesting, a young female 48 years old was diagnosed with acute myeloblastic leukemia. Four years ago when she was 44 years old, she donated for her HLA identical sister with acute myeloblastic leukemia.
G-CSF is safe and very effective for PBSC mobilization in our group of healthy donors. This method allows certain collections of sufficient numbers of progenitors in virtually all healthy donors. We demonstrated that filgrastim mobilization for peripheral blood stem collection is effective and result with successful engraftment in all the recipients. Daily injection of 10µg/kg of G-CSF and first aphaeresis preformed at day 5 seems to be the best strategy to obtain the CD34+ cell count for an allogeneic hematopoietic stem cell graft.

Chronic leukaemia

P1147
Allogeneic haematopoietic stem cell transplantation in patients with myelofibrosis or acute myeloid leukaemia secondary to a previous polycythaemia vera or essential thrombocythaemia: report from the MDS subcommittee of the Chronic Leukaemia Working Party
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Background and aim of the study: The indolent clinical course of Polycythaemia Vera (PV) and Essential Thrombocythaemia (ET) may turn into an aggressive phenotype due to progression to Myelofibrosis (MF) or Acute Myeloid Leukaemia (AML). These patients may eventually become eligible to allogeneic hematopoietic stem cell transplantation (alloHSCT).
Patients: We analyzed 250 patients (male/female 137/113) with an initial diagnosis of PV (n=120) or ET (n=130) who underwent alloHSCT due to progression to MF (n=193) or AML (n=57) and were reported to EBMT registry between year 1994 and 2010. The median age was 56 years (range, 22-75). Of these 250 transplants, 80 were performed after a standard myeloablative and 170 after a reduced intensity conditioning regimen. Donors were HLA related matched (n=115) or mismatched (n=2), or unrelated matched (n=124) or mismatched (n=9). At transplant, 137 (55%) patients had a relapsed/progressive disease, 65 were untreated, 23 were reported as being in CR while the haematologic status was unknown for 25 patients. GVHD prophylaxis was based on Cyclosporine A in 179 cases (72%) either alone (3%) or combined with Methotrexate (34%) or Mycophenolate (35%). A T cell depletion was performed in vivo with ATG (n= 134, 54%) or Alemtuzumab (n= 25, 10%) or ex vivo (n=7, 2.8%).
Results: With a median follow-up of 13 months (range, 1-123), 3 years overall survival (OS), cumulative incidence of relapse/progression (CIR) and transplant related mortality (TRM) were 55% and 32% and 28%, respectively. Acute GvHD grade II-IV was seen in 27% and extensive chronic GvHD in 18% of the patients. When considering factors influencing the clinical outcome after transplant, older age (> 55 years), diagnosis at transplant (AML vs MF) and donor type (mismatched vs unrelated vs related) proved to be associated with a significantly worse outcome (Table 1). Other factors including initial diagnosis (PV/ET), time from initial diagnosis to transplant (> vs < 10 years), JAK2V617F mutation, patient/donor CMV status, disease status at transplant, intensity of the conditioning regimen, stem cell source and T cell depletion had no impact on clinical outcome.
Conclusions: AlloHSCT confirms its curative potential for end-stage PV/ET patients progressing to MF or AML. Relapse and transplant related mortality remain unsolved problems for which innovative treatment approaches are urgently needed.

Table 1
Main clinical outcomes evaluated at 36 months after transplant

Variable	N	CIR (%)	p	TRM (%)	p	OS (%)	p
	250	32		28		55	
Age							
< 55	114	27	ref	20	ref	65	ref
> 55	136	39	0.047	35	0.032	47	0.015
Diagnosis at transplant							
AML	57	53	ref	29	ref	28	ref
MF	193	28	0.001	27	0.045	62	<0.001
Donor type							
Related	115	35	ref	18	ref	65	ref
Unrelated	124	30	0.562	34	0.034	50	0.085
Mismatched	11	35	0.775	49	0.343	30	0.390

P1148
Improved outcome of transplant in patients with CML
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We have evaluated the outcome of 185 patients who received an allogeneic transplant for CML since 2002 in our centre. 124 patients of these pts (67%) were transplanted in 1st CP of CML from a HLA ident. URD (n=70) or SIB donor (n=54). All pts failed or did not tolerate treatment with a tyrosine kinase inhibitor (TKI). Pts transplanted from a SIB donor had a 5-year estimate for OS of 87% (median age 40, pre-transplant EBMT score at median 2) and pts transplanted from an URD had a 5-year estimate for OS of 80% (median age 40 years,EBMT score at median 3). All pts received a myeloablative conditioning regimen including TBI.
The incidence of acute GVHD grade 2-4 was 70.2% for pt transplanted in 1stCP from URD. Further, 77% of these pts developed a chronic GVHD. Haematological relapse occurred in 11 pts from which all except one could be successfully treated with DLI, interferon or TKI. The majority of patients (74.5%) who were transplanted from HLA ident. SIB donor received a graft with highly enriched CD34+ cells without any posttransplant immunosuppression but with a programmed T cell add-back as adoptive DLI. Acute GVHD grade 2-4 occurred in 30.1% of pts transplanted from a HLA-ident.SIB donor and 38.2 % of these pts developed a chronic GVHD. 38 of 42 pts transplanted with CD34+ stem cells received adoptive DLI with or without TKI or/and IFN alfa due to the occurrence or persistence of molecular relapse. Only 5 of these pts developed a haematological relapse from whom 3 pts were retransplanted from an alternative donor.
Further, we evaluated 61 pts who were transplanted for CML in more advanced disease phase with various donor types with an EBMT score at median of 5 (range 2-7). From these pts 55.7% (n=34) were transplanted in second or third chronic phase, 23%

(N=14) in acceleration and 21.3% (n=13) in a manifest blast crises of CML. The 5-year estimates for overall survival declined with increasing EBMT pretransplant risk score as expected. Pts with advanced disease phase of CML had an overall survival of 55%, 45.5% and 15.4 %, respectively. Acute GVHD occurred in 51.8% of all pts, whereas a hematological relapse occurred in 18% 22% and 71.5%, respectively.
In conclusion, for CML pts the results of allogeneic transplant improved in the recent years further and transplant remains especially for pts with low pretransplant EBMT scores, a highly effective 2nd line alternative therapy option after treatment failure of TKI.

P1149

Chronic GvHD is the most pivotal factor for improved relapse-free survival in patients with myelofibrosis after myeloablative allogeneic haematopoietic stem cell transplantation

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Primary or secondary myelofibrosis is a chronic myeloproliferative stem cell disorder which is curable exclusively by allogeneic HSCT.
We report on 69 patients (pts) (37 male, 32 female; median age= 50 years) with primary (n=44), post-polycythemic (n=12) or post-thrombocythemic (n=13) myelofibrosis, who underwent allo-HSCT after myeloablative conditioning with fractionated total body irradiation (TBI) + fludarabine or Cyclophosphamide (n=46) or Treosulfan-based (12-14 g/kg) regimen (n=23). Donors were HLA-identical (n=26) or mismatched (n=3) siblings and matched (n=29) or mismatched unrelated (n=11). Transplants consisted of unmanipulated peripheral blood stem cells (n=62), bone marrow (n=5), 2 pts received highly purified CD34+ cells. GVHD-prophylaxis was performed with CSA + MTX (n=44), in 25 pts. anti-thymocyte-globulin (ATG) (n=12) or alemtuzumab (n=13) was used.
Median follow-up was 25 months, TRM 25%, primary graft-failure occurred in 2 pts (3%). At 3 years, relapse-free survival (RFS) estimates were 52% for all pts, 26% for advanced (n=31) vs 67% for non-advanced (n=38) disease stages (p=0,003). Estimated RFS was 70% for chronic GVHD pts. (n=42) at 3 years vs 22% for pts. without cGVHD (p<0,001). Non-advanced pts. with cGVHD had 82%, advanced pts. with cGVHD 57% probability of RFS at 3 years. Relapse-rate of 17% was significantly influenced by absence of cGVHD (p=0,032) and the use of ATG or alemtuzumab for immunoprophylaxis (p<0,001). No significant influence on posttransplant overall survival could be detected for graft source, donor type, gender constellation, Lille score, grade of marrow fibrosis, recipient age < or > 40 years, JAK2 mutation, splenectomy (SE), conditioning or use of ATG/ Alemtuzumab for immunosupression. Mismatch-transplantation and cytoreductive medical pretreatment (n=38) were associated with impaired relapse-free survival (p=0,042 and p=0,001). SE had no significant impact on the time to leukocyte engraftment.
Our data demonstrate good long-term disease-free survival probability even in advanced disease stages provided that chronic GVHD develops.

P1150

The survival after allogeneic stem cell transplantation for blast crisis chronic myelogenous leukaemia relies on disease status prior to transplant and on the EBMT score, in the tyrosine kinase inhibitors era

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This retrospective national study analysed the impact of BC treatment between 2000-2008 prior to allogeneic SCT, on outcome: 46M and 17F were identified [median age at Tx, 34 (3-63)], with a median interval BC-Tx 19.6 (2.3-113) Mo., 57% of pts were in chronic, 5% in accelerated phase and 38% in BC at diagnosis. Thirty-six % of pts received 1 line, 41% 2 lines, 22%≥3 lines of treatment before BC. For BC, 39% pts had chemo. without TKI and 46% had TKIs±chemo. (6 chemo+dasa, 4 chemo+ima, 8 das alone, 1 dasa and ima, 9 ima, 9 unknown). At Tx 55% were in CR/2nd CP and 45% still in BC, 67% had an identical sibling donor, 2% MM related, 11% matched unrelated, and 20% MM unrelated, 89% pts had a standard conditioning regimen, 11% pts a RIC regimen, 52% had BM cells, 38% PBSC and cord 10%, 22% Tx were sex-MM (F donor/M recipient). The EBMT score was low (1+2+3) for 55% pts, intermediate (4) for 22% pts and high (5) for 14% pts, (and 6) for 5% pts, (undetermined 2 pts). The median FU since Tx was 25 (0.43-109) Mo., 65% of pts had an aGVHD (29 gr I-II, 12 gr III-IV) with no statistical differences between cell sources (p=0.4 for cord vs BM, 0.58 for PBSC vs BM). The CI of cGVHD (14 extensive, 10 limited.) was 43% at latest FU, more frequent in RIC. The CI of CML relapse was 38% at 2 yrs and multivariate analysis demonstrated the favourable impact of disease control prior to Tx [HR 2.22 (1-4.93, p=0.05] with no impact of TKIs on BC treatment and of other variables. The median OS was 22 Mo. The TRM was 33% at latest FU, and was correlated to the EBMT score (p=0.0077 for scores ≥4), and to CML diagnosis in BC (p=0.04). The OS was not improved with standard conditioning regimens, but was by disease control at Tx (p=0.01) and Tx with cord and BM sources over PBSC (p=0.011). In a multivariate analysis, OS was adversely influenced by EBMT score≥5 (HR=4.63 EBMT 5, and 4.77 EBMT 6), BC treatments had no significant impact (with or without stratification on chemo.) on OS and PFS. The median PFS was 5.8 Mo., was not improved by standard conditioning regimens but was improved by disease control prior to Tx (p=0.001) and with cord and BM sources over PBSC (p=0.057). PFS was adversely influenced by EBMT score≥5 (HR=5.82 EBMT 5, and 3.82 EBMT 6), but not BC treatments (±TKIs) prior to Tx. This suggests that OS and PFS are significantly improved when compared to the pre-TKI era, however, the use of TKIs alone or combined to chemotherapy does not seem to influence Tx results yet.

P1151

Evaluation of allogeneic haematopoietic stem cell transplantation and pre- and post-transplant therapy in high-risk patients with chronic myeloid leukaemia

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The introduction of the tyrosine kinase inhibitor (TKI) imatinib for chronic myeloid leukemia (CML) pts led to a rapid decline in allogeneic haematopoietic stem cell transplantations (HSCT) in first chronic phase (CP1). TKIs have been introduced in the pre- and post-transplant period. We evaluated the outcomes of HSCT, and of pre- and post-transplant use of TKIs in all CML pts in our centre from 2002-2009. There were 68 patients (m/f =39/29), including 7 children (median, 44 yrs; 9 – 71): 27 CP1, 41 >CP1 (26 advanced CP, 7 AP, 8 BP) (Table 1). Between 2002 and 2009, annual HSCT rate for CML dropped from 23 to 5 (22% of initial rate). The proportion of advanced disease increased from 48% to 80% of all CML-HSCTs in 2009. Pre-HSCT TKI use increased from 9/23 (39%) in 2002 to 5/5 (100%) of all patients in 2009, while the average annual post-transplant TKI use increased from 18% (2002-2004) to 62% (2007-2009). Pts received PB/BM (n = 58/10) from related/unrelated donors, under myeloablative/reduced-intensity conditioning (MAC/RIC =45/23). Overall, 48 patients received TKIs pre-HSCT, and 19 post-HSCT, for an average of 17 ± 19.4 months pre-HSCT, and 20 ± 16.8 months post-HSCT. The total duration of TKI use in these patients was 24 ± 23.5 months. Grades II-IV acute GvHD occurred in 25 (37%) pts. 43 pts (63%) were alive after a median follow-up of 28.4 months (0.8 – 103.4 mo); with 25 deaths from transplant-related (n=10), CML-related (n=9), or unknown (n=6) causes. 17 pts (25%) developed post-transplant relapses. Overall survival (OS) of all CP1 pts achieved a plateau of 85% at 10 months, and was significantly better compared to advanced disease (>CP1, AP, BP) who had an OS of 65% and 47% at 1 and 2 years (p=0.002). Relapse-free survival (RFS) was 85% at 1 and 2 years, and 81% at 5 years. Pts who had HSCT in CP1 had OS and RFS advantage over others (p=0.002 and 0.001, Figure 1). There was no statistical difference in OS or RFS of RIC versus MAC pts. Despite the decrease of HSCT rates for pts in CP1, HSCT rates remained stable for patients in advanced CML over the past decade in this monocentre study. TKI use before and after HSCT became standard, but despite that and the introduction of RIC, transplant outcomes for these patients remain critical. Thus, research should focus on the improvement of conditioning and the standardization of TKIs use for pts in advanced CML in the post-transplant period.

P1152

Pgp-mediated resistance toward both imatinib and NK-cells killing of aggressive minor CML blast subset can be reversed by Pgp modulators

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Objectives: In advanced CML, imatinib treatment and immunotherapy via NK-cells are less efficient than in CP-CML. We have previously found that in advanced CML, blasts of the same CML clone are heterogeneous, containing a minor subset

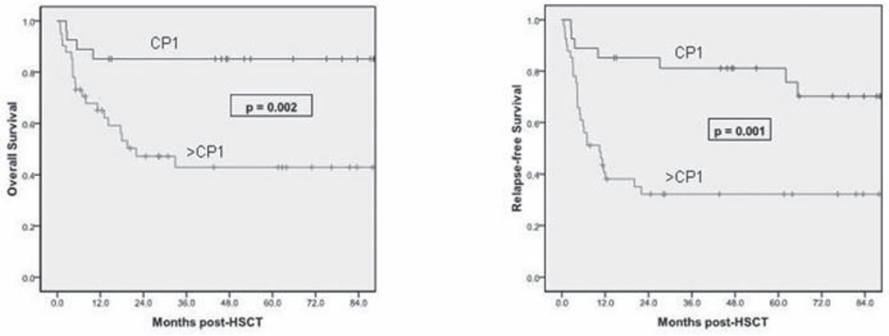
[P1151]

Table 1. Patients' and donors' characteristics

Variables	Patients	Donors
	No. (%)	No. (%)
Gender: male/female	39/29 (57/43)	48/20 (71/29)
Gender match: yes/no	45/22 (67/33)	
Disease phase at SCT: CP1/>CP1	27/41 (40/60)	
Hematologic remission: CHR/<CHR	46/22 (68/32)	
Cytogenetic remission: CCR/MCR/<MCR	14/9/36 (24/15/61)	
Additional chromosomal changes: yes/no	18/50 (26/74)	
HLA type match: yes/no	59/9 (87/13)	
Related/unrelated		23/45 (34/66)
Stem cells: PB/BM		58/10 (85/15)
CMV status pat.don: pos.pos/pos.neg/neg.pos/neg.neg		31/7/5/23 (47/11/8/35)
GvHD prophylaxis: CsA + MTX/MMF	59/9 (87/13)	
ATG Fresenius/TG Mérieux	35/17 (67/33)	
Conditioning regimen: MAC/RIC	45/23 (66/34)	
Bu-based/TBI-based	61/7 (90/10)	

CP1, first chronic phase; CHR, complete hematologic remission; CCR & MCR, complete & major cytogenetic remission; HLA, human leucocyte antigen; PB, peripheral blood; BM, bone marrow; CMV, cytomegalovirus; pat, patient; don, donor; pos, positive; neg, negative; CsA, cyclosporin A; MTX, methotrexate; MMF, mycophenolate mofetil; ATG, anti-thymocyte globulin; TG, thymoglobulin; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; Bu, busulphan; TBI, total body irradiation.

Figure 1. Overall survival (OS; on the left) and relapse-free survival (RFS; on the right) following HSCT comparing patients in CP1 and advanced CML (>CP1) following HSCT.



(MS) of blasts that are significantly more aggressive than the common subset (CS) [Differentiation. 76: 908-922, 2008]. We aimed this study to investigate whether the MS blasts exhibit differential resistance mechanisms toward imatinib and NK-cells mediated killing.
Methods: To this end, we have compared the two blast subsets for the level of resistance to imatinib and for their sensitivity towards NK-cells in relation to expression of a functional P-glycoprotein (Pgp, an ABCB1 multidrug transporter). The killing ability of healthy donor NK-cells and NK-92 cell line toward the two subsets was measured by standard cytotoxicity assay. The mechanism of Pgp involvement in the interaction of the malignant cells with NK-cells was also studied using genetically engineered Pgp-TET-ON/OFF (conditional expression) cellular system.
Results: While Pgp could not be detected on the cell surface of the CS blasts, Pgp is exclusively highly expressed in the MS blasts. Functional Pgp assays in the MS blasts indicated unequivocally that imatinib is a substrate for Pgp. As imatinib efficiently inhibited the proliferation of the CS blasts in a dose-dependent manner, the proliferation rate of the MS blasts was essentially not affected. Similarly, the MS blasts were significantly less affected by NK cells than the CS blasts (2.2±0.2 -fold, p<0.001). Further analyses indicated that the Pgp-expressing MS blasts become mainly less susceptible to the TRAIL-mediated apoptotic killing pathway of NK cells. Interestingly, both the anti-proliferative effect of imatinib on the MS blasts and their sensitivity to killing by NK cells could be restored by addition of the Pgp inhibitor R-VRP, in a dose-dependent manner.
Conclusions: The existence of a minor subset of CML blasts of both greater clonogenicity and high expression and activity levels of Pgp, apparently signify clonal evolution toward both increased malignancy and lower therapeutic sensitivity to both imatinib and NK cells. Moreover, this study suggests that combination therapies with Pgp-modulators might also be clinically effective in targeting the MS aggressive blasts.

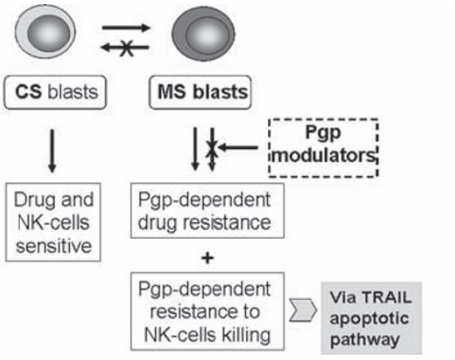


Figure1: Differential properties and targeting of the aggressive CML blast subset

P1153
Chronic phase CML patients on tyrosine kinase inhibitors have a normal cellular immune response against influenza but an impaired IgM humoral response against pneumococcus after vaccination: implications for immunotherapy
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Imatinib, nilotinib and dasatinib are remarkably effective as single-agent treatments for chronic myeloid leukemia (CML) in chronic phase (CP). They are also increasingly being used as therapies in the allogeneic stem cell transplantation (allo-SCT) setting, both as post allo-SCT maintenance treatment for Ph+ leukemias as well as for their putative immunomodulatory effects. However little is known on their potential impact on the immune system and to date no human in vivo data are available. To characterize the in vivo immunomodulatory effect of TKIs, 50 CP-CML patients on standard dose tyrosine kinase inhibitors (TKIs) and 15 healthy controls were vaccinated against Flu and pneumococcus at our institution. Samples were taken pre and at 1 and 3 months post-vaccination. Titers of IgM and IgG anti-pneumococcal were determined using ELISA technology, and the proportion of B-cell subsets (IgM memory and switched memory B-cells) were measured using flow cytometry. We analyzed the immunological T-cell response to influenza virus both quantitatively and qualitatively using flow cytometry for intracellular TNF-α, IFN-γ, IL2 and the cytotoxicity marker CD107a.
We found that a significantly lower proportion of patients mounted an anti-pneumococcal IgM response at 4weeks post vaccination (defined as IgM serum titer > 80 U/ml and 4-fold increase from baseline) compared to healthy controls (20/45 versus 11/12, p=0.004). The median percentage of IgM memory B cells in controls was 13.9% (range 7.06 to 18.45); the frequency of IgM memory B cells was below this range in 17 out of 36 patients (Fisher exact test, p=0.004). In contrast, CD8 and CD4 T cell responses to Flu vaccination were not significantly different between patients and controls. Prior to vaccination, T cell responses against Flu were detected in 11/33 patients on TKI and 2/10 healthy controls, indicating pre-existing memory T cell responses to Flu. After vaccination this proportion increased to 19/32 in patients on TKI and 8/10 in healthy control.
These data have significant implications for the use of TKI in conjunction with allografting and immunotherapy. Whereas TKI do not appear to impair the cellular immune response to viruses, we found a significant immunomodulatory effect on the IgM antibody response to pneumococcus associated with a selective reduction in the IgM memory B cell subset. We are currently investigating the mechanism underlying this observation.

P1154
RIC-SCT in 41 consecutive CLL patients from a single centre: unrelated donor provides better disease control with comparable non-relapse mortality
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Despite innovative treatments CLL remains essentially incurable disease. Alternative approach is particularly needed for pts with an accumulation of adverse prognostic factors (unmutated IgVH, del17p, purine resistance). AlloSCT with reduced conditioning (RIC) is promising therapeutic alternative because it reduces toxicity whilst preserving GVL. Availability of related donor is limited and due to the higher TRM there was a reluctance to use unrelated donors (URD). Recent improvements in typing techniques and supportive care have made the use of URDs a realistic choice.
Patients & Methods: Unicentric retrospective analysis of 41 consecutive pts with CLL (median 60 y, range 48-70) transplanted between 2002-9 by RIC protocols (Cy-Flu, Flu-Mel) either with sibling (SIB group, n=16,39%) or unrelated donor (MUD, n=25,61%). Cytogenetics was available in 39 (95%) pts with unfavorable in 23 (59%). Del17p was detected in 15 (38%) of pts. IgVH status was known in 17 patients (41%) with 15 (37%) being unmutated. Pts received a median of 3 lines of therapy (1-6) before SCT, all were refractory to purines. 20 (41%) were transplanted outside of CR/PR. There were no significant differences between SIB and URD group with respect to relevant parameter (age, stage, cytogenetics, IgVH). URDs were significantly younger than the SIBs (median 29 vs.56, p=<,0001).
Results: For all patients (median follow of 22 mts, range 3-77) the 3-y Kaplan-Meier OS/DFS probabilities were 41% and

39%, respectively. Nonrelapse mortality was 41% (17/41) and relapse incidence 22% (9/41).The overall incidence of aGVHD and cGVHD was 56% and 48%. There were no differences in OS/DFS between patients with and without del17p, as well as between patients with unfavorable cytogenetics and others. We reported similar 3-y OS (32% vs 55%, p=0.42) for URD and SIB group, but URD experienced significantly improved DFS (23% vs 55%, p=0.03). This was due to higher incidence of relapse (44% vs 8%, p=0.06) and significantly higher incidence of graft rejection (38% vs 0%, p=0,0018) for the SIB. On the contrary there was no difference in NRM (31% vs 40%, p=0,41).The incidences of gr. III-IV aGVHD and cGVHD were also similar (SIB/URD 13% vs 8%, p=0,64 and 29% vs 63%, p=0,08).
Conclusion: RIC SCT is an effective treatment of poor prognosis CLL. It can be curative even in pts refractory to purines or with del17p. URD has no adverse impact on SCT and is a viable choice with probably better disease control.

P1155

Staging criteria based on maximal disease stage pre-transplant predicts the outcome of allogeneic stem cell transplantation for chronic myeloid leukaemia in the imatinib era

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Several disease phase classification systems, all of which were designed in the pre-imatinib (IM) era are used for CML. These systems have not been validated for determining the outcomes of allogeneic stem cell transplantation (SCT) in the IM era. We propose a set of 'modified Hammersmith criteria' (Savage et al 1997) that further distinguishes advanced phase (Adv) patients based on maximal disease phase (i.e. blast phase (BP) at any stage during the disease history), irrespective of whether a second chronic phase (CP2) was restored after chemotherapy or tyrosine kinase inhibitors (TKI) prior to SCT. We compared the outcome of patients undergoing SCT after failure of therapy with TKI according to the modified Hammersmith criteria, assessed before transplant, with outcomes using 3 other classification systems (CIBMTR, Kantarjian and WHO). From 1/2000 to 12/2009, 69 CML patients in all stages were treated with TKI (53 IM only, 10 IM followed by dasatinib (DAS), 3 IM followed by nilotinib (NIL) and 3 IM followed by DAS and NIL). The median age was 40.8 years; 49 patients were transplanted from matched unrelated donors and 53 received myeloablative conditioning. Indications for SCT included suboptimal response or failure to TKI, progression from CP to accelerated phase (AP) or BP, presentation in AP or BP. The 3-year probabilities of survival (OS) according to the CIBMTR criteria were 67% for 14 CP patients compared to 54% for 13 AP, 0% for 3 BP and 51.7% for 29 CP2 patients P=0.085. Using WHO criteria, the 3 year OS were 84.2% for 20 CP patients compared to 43.8% for 25 AP patients, 0% for 3 BP patients and 42% for 21 CP2 patients, P=0.002; the corresponding values using Kantarjian criteria were 77.1% for 23 CP patients compared to 0% for 5 AP, 0% for 1 BP, 55.3% for 26 CP2 and 35.7% for 14 CP with clonal evolution, P=0.001. In contrast, employing the modified Hammersmith criteria, the 3-year OS rates were 79.3% for 20 CP, 55.9% for 27 AP and 17.2% for 22 patients who had been in BP at any stage in their disease, irrespective of whether CP2 was restored (P<0.0001). These data suggest that the modified Hammersmith classification that takes into account the "maximal disease phase" during the evolution of the leukaemia pre-transplant as well as the actual phase at the time of the transplantation can accurately assess the prognosis of patients after SCT. Patients in CP2 after prior BP may be candidates for 'maintenance' with TKI after SCT.

P1156

Allo HSCT with RIC for poor-risk CLL in Sweden: donor T-cell engraftment 3 months after transplantation predicts long-term survival

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Allogeneic hematopoietic stem cell transplantation (alloSCT) after reduced-intensity conditioning (RIC) is a potentially curable treatment option for eligible patients with poor-risk chronic lymphocytic leukemia (CLL). Thirty-eight adult patients with CLL underwent RIC alloSCT in Sweden between 1999 and 2007. Early mortality at day +100 was 10%. The cumulative incidence of acute GVHD grades II-IV and chronic GVHD was 29% and 48%, respectively. Four patients (11%) developed post-transplant lymphoproliferative disease after a median of 102 days (range 42–211); one of them died. Non-relapse mortality at 1, 3 and 5 years was 18%, 21% and 21% respectively. Progression-free survival (PFS) at 1, 3 and 5 years was respectively 47%, 33% and 25%, while overall survival (OS) 1, 3 and 5 years after transplantation was 74%, 58%, and 45%, respectively. Seventeen patients achieved >90% donor T-cell engraftment at 3 months after alloSCT and, as compared to the patients with ≤90% donor T-cell engraftment, they showed favorable PFS at 1 year (82% vs 21%, P=0.001), and better long-term PFS and OS (P=0.001 and 0.02 respectively). Donor T-cell engraftment >90% at 3 months after RIC alloSCT for CLL seems to be a promising predictive value of both short-term and long-term PFS and OS.

P1157

Oxidative stress induced by small interfering RNA against glutathione reductase transcripts sensitizes mutated T315I cells to nilotinib

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Introduction: Nilotinib, a second-generation BCR-ABL kinase inhibitor, has become standard for the treatment of CML patients who are either resistant to or intolerant of Imatinib. But Nilotinib failed to overcome the Imatinib resistance caused by the T315I mutation. These findings underscore the importance and urgency to develop alternative strategies. Recent studies provide a rationale for the incorporation of glutathione-modulators in CML treatment, but wide experiences are missing. Therefore, we evaluated specific glutathione reductase small interfering RNA (siRNA) silencing in BCR-ABL positive cell lines, including those with the T315I mutation.
Material and methods: The effects of glutathione reductase siRNA or the combination of glutathione reductase siRNA and Nilotinib were compared to those of Nilotinib on the BCR-ABL gene expression measured by real time reverse-transcriptase polymerase chain reaction analysis in wild-type and mutated BCR-ABL cells (T315I cells). For assessment of cell viability and cell proliferation in each case a MTT assay or a BrdU assay was performed.
Results: The single use of glutathione reductase siRNA has shown no significant effects on the BCR-ABL gene expression in wild-type and mutated BCR-ABL cells compared to control (wild-type p>0.3, T315I p>0.5). Coadministration of glutathione reductase siRNA with Nilotinib dramatically reduced BCR-ABL gene expression in wild-type cells versus control (p<0.001) and versus Nilotinib alone. Cotreatment of glutathione reductase siRNA and Nilotinib resulted also in a significant decrease of BCR-ABL gene expression compared to control (p<0.03) and to Nilotinib (p<0.02) in T315I cells.

Further, nitrosative stress mediated by SperminNONOate induced also a reduced BCR-ABL expression alone and in combination with Nilotinib compared to control (p< 0,002) in wild-type cells. Cotreatment with glutathione reductase siRNA and Nilotinib resulted in a significant decrease of cell proliferation (p<0.03) and cell viability (p<0.03) as compared to Nilotinib alone in T315I cells.
Conclusion: Oxidative stress induced by siRNA against glutathione reductase sensitizes and promotes the drug-sensitivity to Nilotinib in mutated T315I cells. This might be a novel choice for leukemia treatment.

P1158
Osteopontin expression on the endosteum correlates with the extent of fibrosis in primary fibrosis and decreases post-haematopoietic stem cell transplantation
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Objectives:The hallmarks of primary myelofibrosis (OMF) is a high degree of positivity of a reticulin and collagen staining. Bone trabecules are usually thickened with numerous osteoblasts at the margin of the osseum. Osteopontin (OPN) was detected in several fibrotic processes including liver and cardiac fibrosis. Therefore, we investigated the expression of OPN on bone trabecules in the trephine biopsy specimens in the OMF patients before and after hematopoietic stem cell transplantation (HSCT).
Methods: OPN expression was examined in trephine biopsy of 11 OMF patients (9 females, 2 males age 29–57 yrs). All received allo HSCT (7 sibling, 4 matched unrelated donors). The effect of transplantation was evaluated at the level of trephine biopsies before and 1mo, 6 mo and 1 year after HSCT. One patient was in stadium 1, 6 stadium 2, and 4 stadium 3, according to the Kiel criteria. From the clinical onset of the disease to HSCT was 7 to 52 mo (median 19).Two patients received myeloablative, 9 reduced intensive conditioning. All patients (except one) were transplanted with PBPC with CD34 dose 1.8 to 11.7x 10^6 /kg b.w (median 6.63). Efficacy of HSCT was evaluated by the presence of donor chimerism using PCR-STR method. All patients were assessed for the presence of reticulin fibers and collagen bunches, and by immunocytochemistry for the OPN expression before and after HSCT.
Results: The median observation was 42 months. Three patients died from 1 to 3 months post HSCT. All other were observed for at least one year. Haematological reconstitution was seen by 15 days after HSCT. All alive patients were full chimera.
Trephine biopsy: reticulin fibers and collagen bunches and elevated number of megakariocytes were seen in all cases. Immunocytochemistry documented abundant expression of OPN in the endosteum of the bone trabecules documenting a high degree of osteoblasts activation. In the 1-year follow-up after HSCT, OPN was not seen as before, as a thick and wide area and became linear along the trabecules boundary. This progressive reduction of OPN expression over the time post HSCT correlated well with higher representation of the hematopoietic tissue.
Conclusions: Fibrotic process in OMF is associated with an abundant OPN expression in the endosteum of the bone trabecules. HSCT is curative procedure in majority of OMF cases. The resolution of clinical symptoms of the OMF after HSCT associates well with the lowering of OPN expression.

P1159
Allogeneic stem cell transplantation in myelofibrosis; a single-centre experience
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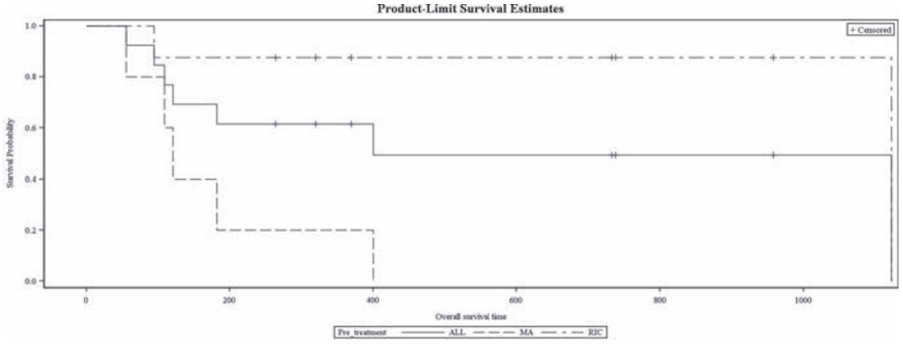
Background: Allogeneic stem cell transplantation (alloSCT) is a potentially curative treatment option for patients with myelofibrosis (MF; Merup et al. 2006, Kröger et al. 2009, Bacigalupo et al. 2010, Stewart et al. 2010). Reduced-intensity conditioning (RIC) may be the best choice for the majority of patients up to the age of 70 years.
Aim of the study: This analysis of prospectively collected data of allotransplanted patients with MF aimed at clarifying the outcomes of alloSCT at a single Finnish transplant center.
Patients: A total of 13 patients with MF underwent 14 alloSCTs between years 1999 and 2009 (blood SCT 12 and bone marrow transplant 2). The median age was 58 (22-64) years. The median time from diagnosis to transplantation was 24 months (5-165) and from treatment onset 18 (5-163) months. Lille scores of 0/1/2 at transplantation were 2/7/4, resp. The donor was sibling (3) or MUD (11), and conditioning was myeloablative (MA; n=5) or RIC (9). The median number of infused CD34+ cells was 5.6 (2.1-16.7) x 10^6 cells/kg. The outcome analyses are as of July 2010.
Results: Seven patients had severe transplant-related complications: sepsis (n=3), grade 3-4 acute graft-versus-host disease (GVHD; 2), post-transplant lymphoproliferative disorder (1), extensive chronic GVHD (1) , veno-occlusive disease (1), and haemolytic uremic syndrome (1). The transplant-related mortality (TRM) at 1 and 3 years was 80 and 100 % for the patients with MA conditioning vs 13 and 25 % with RIC . The type of conditioning appeared to be the only significant predictor of TRM(P=0.004). At one year after RIC transplantation, 75 % of patients were in CR, and the respective figure at 3 years was 38 %. At the time of analysis, four patients (31 %) had a continuous CR. The survival curves for all patients and the MA and RIC cohorts are shown in Fig.
Conclusion: AlloSCT is a potentially curative treatment for MF, but the choice of patients and conditioning intensity are critical for the transplant outcomes. The high TRM of MA conditioning can be avoided by RIC, and long-term results after RIC transplants seem promising.

[P1159]



P1160

Circulating CD34+ cells as prognostic and follow-up marker in patients with myelofibrosis undergoing allogeneic stem cell transplantation

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Objective: Elevated circulating CD34+ cell count in peripheral blood (CD34+PB) is a hallmark of primary myelofibrosis (PMF) but its prognostic impact is controversial.
Patients and methods: We examined the prognostic value of CD34+ PB in 59 patients (n=38 PMF, n=18 post ET/PV MF and n= 3 AML) before and after reduced-intensity allogeneic stem cell transplantation (ASCT).
Results: A weak negative correlation was found between myelofibrosis duration and CD34+PB (r=-0.29, p=0.03). CD34+PB tended to be higher in post-PV/ET MF compared with PMF (p=0.07) and proliferative disease phenotype compared with depletive phenotype (p=0.009). CD34+PB neither influenced overall (OS) and disease-free survival (DFS) nor predicted risk of relapse post-ASCT. During the follow-up post-ASCT, CD34+PB were elevated only in 1 out of 8 patients with molecular relapse or minimal residual disease and 3 out of 9 with clinical relapse.
Conclusion: CD34+PB correlate with disease phenotype, duration of myelofibrosis and post-PV/ET MF. No certain prognostic relevance could be attributed to CD34+PB, and the value in predicting relapse was only weak in comparison to JAK2V617F-PCR and chimerism.

P1161

Role of 18F-FDG PET-TC in evaluating response to reduced-intensity conditioning allogeneic transplant in heavily pre-treated patients with chronic lymphocytic leukaemia: preliminary results in 9 cases

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Chronic lymphocytic leukemia (CLL) is a heterogeneous lymphoproliferative disorder; the only potential curative option especially for high-risk patients is the allogeneic transplant. To our knowledge, no studies have been published on the role of 18F-FDG PET or PET-CT in evaluating response to allogeneic transplant in CLL.
The aim of our study was to evaluate the role of 18F-FDG PET-CT in monitoring response to reduced-intensity conditioning (RIC) transplant and to compare the results with standard criteria according to International Workshop on Chronic Lymphocytic Leukemia (IWCLL).
We retrospectively analyzed 9 consecutive high-risk CLL patients, affected by fludarabine-refractory (4 patients) or relapsed CLL (5 patients) who underwent RIC transplant from March 2004 to May 2009. PET-CT scanning was planned at about 8 months after transplant to assess response and at a mean 9 month period during follow-up. Overall, 42 PET-CT studies were performed. The mean long-term follow-up period was 39.7 ± 20.5 months. All studies were analysed qualitatively and scored as either negative or positive: any focal area of activity higher than vascular background, in any site incompatible with normal anatomy, was considered abnormal.
The first PET-CT performed after transplant showed abnormal FDG uptake in 5 patients: 4 patients classified as stable/refractory and 1 patient in partial remission(PR) at pre-transplant evaluation. No abnormal FDG uptake was observed in 4 patients who showed PR before transplant. Response assessment by IWCLL criteria performed at the same time of the first PET-CT (at about 8 months after transplant), showed persistent disease in 8 patients (5 PET positive and 3 PET negative) and complete response in 1 patient (PET negative).
At the end of follow-up, all 4 patients with previously negative scans were still PET negative and are in complete remission by standard criteria. Similarly, all 5 patients with previously positive scans were still PET positive; of these, 1 died 27 months after RIC transplant for disease progression and 4 are alive with persistent disease. From our preliminary data in a small series of CLL patients, the first 18F-FDG PET-CT after transplant shows different metabolic findings that reflect the different pre-transplant status and seem to predict the patient outcome earlier than clinical evaluation by standard criteria. PET-CT performed during follow-up is useful to assess disease status and to early detect disease progression.

P1162

Prior treatment with dasatinib followed by stem cell transplantation in patients with CML in advanced phases with BCR-ABL mutation

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Objectives: The prognosis for patients with chronic myeloid leukemia (CML) in advanced phases remains dismal, especially for those who develop ABL mutation. Administration of second-generation tyrosine kinase inhibitors (TKI) might provide an effective approach for patients with BCL-ABL mutation, but it only offers short-time benefit in most cases. Allogeneic stem cell transplantation (allo-SCT) seems the only curative therapy for advanced CML, and limited data exist on the effectiveness of allo-SCT combined with new TKI dasatinib.
Methods: Five patients with BCR-ABL mutations in accelerated phase (n=1) and blast crisis (n=4) were enrolled. All the patients had a history of chronic phase (CP) before enrollment. Three patients progressed to advanced phases during imatinib treatment. The other two patients didn't receive any TKIs during the CP. When progressing to blast crisis, imatinib was administered but showed no response. All the patients received dasatinib for a predetermined period, and then underwent busulfan/cyclophosphamide based myeloablative allo-SCT. ATG was used for those who received HLA-haploidentical transplantation. Cyclosporin, mycophenolate mofetil and short-term methotrexate were used for graft-versus-host disease (GVHD) prophylaxis.
Results:

1. Patients were treated with dasatinib for 1-3months (median, 2 months). At the time of transplant, all patients achieved complete hematological response and returned to CP, and two patients achieved partial cytogenetic response. Dasatinib was well-tolerated expect one patient who developed pleural effusion.
2. Donors were HLA-haploidentical related in two cases, HLA-identical related in one case, and HLA-identical unrelated in two cases, respectively. All patients achieved complete donor stem cell engraftment. Two patients experienced grade III-IV acute GVHD. Chronic GVHD (cGVHD) was observed in three patients, including one with extensive cGVHD. Only one patient died from pulmonary fungal infection while in complete molecular remission. After a median follow-up of 6.5 months, four patients were alive with complete cytogenetic remission, including three in complete molecular remission. The relapse rate after allo-SCT was 0.

Conclusion: Myeloablative transplantation for imatinib-resistant CML may have a satisfactory outcome when combined with dasatinib, which could provide a good quality of remission prior to transplantation and a curable opportunity after transplantation.

P1163

Second-generation TKI prior to HCT in patients with chronic phase CML has no adverse influence on HCT toxicity and outcome

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All chronic myeloid leukemia (CML) Ph(+) patients resistant to imatinib and second generation tyrosine kinase inhibitors (TKI), according to ELN recommendations, should be allo-transplanted. Limited data exist whether second generation TKI given prior to allogeneic hematopietic cell transplantation (HCT) increase transplant-related toxicity and worsen the outcome of HCT.
The outcome of 15 CML patients treated with dasatinib or nilotinib or both before HCT was retrospectively analyzed. At the time of transplant 9 patients were in chronic phase (CP) with no cytogenetic response and 6 patients in blastic phase CML (BP). Among these patients 9 had matched unrelated donors (MUD) and 6 had related donors. Stem cells source was peripheral blood in 13 cases and bone marrow in 1 case. 11 patients received myeloablative conditioning regimen and reduced intensity conditioning (FluMel or MelTreoFlu) was given in 4 patients. All except 1 patient engrafted successfully with median time 20 days (14-43 days). Severe transplant-related toxicity was observed in 2 (13,3%) cases: hepatic veno-occlusive disease (VOD) and multi-organ failure. 33,3% patients experienced acute graft versus host disease (GvHD), among them 2 in IV grade, and in 20% of patients chronic GvHD developed.
Among 9 patients transplanted in CP 6 patients (66,7%) are alive with median follow-up 12 months (2-29 months). Whereas in the group of 6 patients transplanted in BP, 3 died of relapse, 1 because of VOD and 1 due to GvHD in early post-transplant period.
Conclusion: Second generation TKI do not negatively effect transplant-related toxicity and outcome of the patients transplanted in the CP. Very poor outcome observed in the BP CML group of transplanted patients might have been caused by the advance stage of disease rather then TKI-related toxicity.

P1164

Lenalidomide and rituximab in combination with donor lymphocyte infusions show high efficacy in a patient with early relapse of chronic lymphocytic leukaemia after allogeneic stem cell transplantation

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Patients with poor-risk chronic lymphocytic leukemia (CLL) relapsing after allogeneic stem cell transplantation (SCT) have limited therapeutic options and bad prognosis. Whereas for selected CLL patients failing conventional therapy the immunmodulator lenalidomide has shown some efficacy, no data are available for patients after allogeneic transplantation. Here we describe a 43-year old woman with fludarabine-refractory CLL who did not respond to further salvage attempts with rituximab-bendamustin and underwent SCT from a matched unrelated donor (10/10 allele level) while still suffering from resistant bulky lymphadenopathy. After conditioning with fludarabine, busulfan, cyclophosphamide and ATG rapid engraftment could be achieved with no signs of acute GVHD. 8 weeks after transplantation complete donor chimerism was seen, but minimal residual disease monitored by MRD-flow was still positive (0.09%) in peripheral blood. On day +125 peripheral lymphadenopathy and splenomegaly recurred. Despite rapid tapering of immunosuppression no GVHD could be triggered and consecutively systemic massive lymph node enlargement developed within 2 weeks. Lymph node biopsy as well as bone marrow aspiration both showed recurrence of CLL. In the absence of other reasonable therapeutic options, lenalidomide was started (15mg daily days 1-21) together with rituximab 325mg/sqm every 4 weeks. Treatment was very well tolerated, with grade III neutropenia being the only significant adverse event. After 3 cycles PR was achieved and we continued the treatment but added donor lymphocyte infusions (DLI, starting dose 5,1 x 106 CD3+/kg) in escalating doses over the next 5 months. Lenalidomide / rituximab was interrupted during every first 3 weeks after DLI. In the absence of any signs of GVHD, 8 months after start of lenalidomide and 11 months after SCT the patient achieved for the first time complete remission and MRD negativity. She remained a complete donor chimera and currently in continuing CR 10 months after accomplishment of the lenalidomide-rituximab-DLI salvage regimen. No acute GVHD developed and the patient is in very good condition.
Conclusion: Rituximab-lenalidomide-DLI can be highly effective in patients with bulky CLL relapsing after SCT. It remains to be settled, however, whether efficacy of this regimen can be demonstrated in a relevant proportion of patients, and what the contribution of the “allo-effect” in this setting might be.

P1165

Donor lymphocyte infusions but not alemtuzumab induce long-lasting complete remission in T-PLL after allogeneic haematopoietic progenitor cell transplantation

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Objectives: T-cell prolymphocytic leukemia T-PLL is a disease with an unfavorable prognosis. Although introduction of the therapy with the CD52 antibody, alemtuzumab, led to a substantial improvement of remission induction and remission duration cure can usually only be achieved by an allogeneic transplantation (TX) of hematopoietic progenitor cells (HPC). We report a case that clearly demonstrates the superiority of immunological control of T-PLL by T-cells of the HPC donor in comparison to treatment with alemtuzumab.
Case Report: In a 53 years old woman with a chronic hepatitis B virus infection T-PLL was diagnosed in 2006. Initial treatment with chemotherapy (fludarabine, cyclophosphamide, mitoxantrone) and alemtuzumab led to a short-lasting cytological remission. Therefore, a TX with peripheral blood HPC from a matched unrelated donor after conditioning with BEAM (BCNU, etoposide, cytosin-arabinoside, melphalan) and alemtuzumab was performed in May 2007. This led to a cytological and molecular (based on analysis of a patient-specific T-cell receptor β rearrangement, sensitivity of at least 0.01% of analyzed nuclear cells) remission. On day +351 after TX, molecular analysis of PB revealed a relapse at the level of 0.16%. A treatment with alemtuzumab with a dose of 133mg led to a complete cytological and molecular remission. In parallel, on day +383 after TX a first donor lymphocyte infusion (DLI) was given at a dose of 5x10E+05 CD3+ cells/kg of body weight (bw). On day +421 a second DLI with a dose of 1x10E+06 CD3+ cells/kg of bw was given but one week later analysis of the PB revealed again a molecular relapse of T-PLL at the level of 6.5x10E-04. On day +453 after TX DLI was further escalated and given at a dose of 3x10E+06 CD3+ cells/kg of bw and a concomitantly measured MRD level of 3.7x10E-03. The MRD level further increased to 2x10E-02 on day +469 after TX. One week later, the patient developed acute Graft-versus-host disease (GvHD) of the skin and on day +490 post TX no further MRD could be detected in PB. This cytological and molecular complete remission now lasts until day +1361 post TX without further immunological treatment or chronic GvHD.
Conclusion: The presented case demonstrates that relapse of T-PLL after allogeneic TX can be managed and controlled for

long-lasting time periods by administration of escalating dosages of DLI. Such immunological control of T-PLL is obviously superior to treatment with alemtuzumab.

Myelodysplasia

P1166

Immunosuppressive therapy with anti-thymocyte globin and cyclosporine A in patients with refractory cytopenia in childhood

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Objective: Refractory cytopenia in childhood (RCC) is the most common subtype of pediatric myelodysplastic syndrome (MDS). In addition to allogeneic hematopoietic stem cell transplantation (HSCT), immunosuppressive therapy (IST) has recently been used as a treatment modality. Here we report the result of IST with anti-thymocyte globulin (ATG) and cyclosporine A (CSA) in a selected cohort of patients (pts) with RCC.
Patients: Ninety-one pts (52 boys /39 girls) with RCC were given IST as first line treatment. At diagnosis, the median age was 9.8 (1.2-18.1) years. The median absolute neutrophil count (ANC) was 315 (0-3038) x10e9/L and most pts were transfusions dependent for platelets (n=77) and/or red cells (n=66) at the start of IST. Bone marrow cellularity was low in 78 pts and normo/hypercellular in 8 pts. Cytogenetic analysis revealed a normal karyotype in 49 pts, an abnormal clone in 3 pts and no result in 39 pts. IST was started at a median of 67 (0-472) days after diagnosis. Fifty-four pts was given Thymoglobuline® (rabbit-ATG), 33 pts Lymphoglobuline® (horse-ATG), and 4 pts others. The median follow-up after IST was 1070 (14 to 3747) days.
Results: 57 (63 %) pts responded to IST at 6 months (complete response =normal blood count (CR): n=6, partial response (PR): n=51). Rabbit-ATG showed the similar response rate compared to horse-ATG (57.6% vs 68.5% p=n.s.). There were also no differences in response according to age, days between diagnosis and IST and ANC at IST. Late response after 6 months was observed in 7 pts. Clonal evolution occurred in 6 pts (cumulative incidence at 5 years =10.2%) including 3 pts with -7 or 7q- aberrations. Seven responders experienced recurrent disease. One child developed clinical PNH. Thirty-five pts (38%) received HSCT as the second line therapy. The overall and failure free survivals (FFS) at 5 years were 89.5% and 39.7%, respectively. At the time of the last follow-up, 25 pts (27%) had CR and 22 pts PR (24%).
Summary: In this selected patient population with RCC, about 60% of pts responded to IST. A normal blood count was achieved in 27 % of pts. This finding suggests that the immune system plays the key role in the pathophysiology of bone marrow failure in some pts with RCC. However, the FFS was around 40% and has not reached a plateau at 5 years after IST. Pts with RCC after IST continue to be at risk for disease progression and relapse and a considerable number of pts need second line HSCT.

P1167

Is there a graft-versus-MDS effect after haematopoietic allogeneic stem cell transplantation?

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We studied the effect of acute and chronic graft-versus-host on relapse incidence in patients with MDS after allogeneic stem cell transplantation.
Patients: Ninety-one patients with MDS and receiving an allogeneic hematopoietic stem cell transplantation (HSCT) at Karolinska University Hospital between 1992 and 2010 was studied. There were 50 males and 41 females with a median age of 39 years (1-67), 28 (31%) were children <18 years. There were 14 patients considered as low risk and 77 as high risk. Donors were a HLA-identical sibling in 36 cases, HLA-A, -B and –DR identical unrelated (MUD) in 46 cases and a mismatched unrelated in 9 cases. Most patients received CsA and MTX as GVHD prophylaxis. Median nucleated cell dose was 8.1x108/kg. Conventional myeloablative conditioning (MAC) was given to 57 patients and reduced intensity conditioning (RIC) to 34 patients. MAC mainly consisted of Busulfan (Bu) and cyclophosphamide (Cy) while RIC mainly consisted of Fludarabine and Bu. Anti-thymocyte globuline (ATG) was given to 68 patients.
Results: Relapse occurred in 23 patients at a median of 265 days (60-3861). The cumulative incidence of relapse was 24% after 5 years and 29% after 10 years. The cumulative incidences (CI) of relapse in patients with no (n=34), grade I (n=23), II (n=22) or III-IV (n=12) acute GVHD were 35%, 31%, 21% and 17%, respectively (ns). In patients with (n=24) or without (n=56) chronic GVHD, CI of relapse was 25% in both groups. In patients with mild or moderate cGVHD, CI of relapse was 31% and 13% (ns). No combination of acute and chronic GVHD showed any effect on relapse. In multivariate analysis high age (HR 1.36, 95% CI 1.07-1.73, p=0.012) and female donor to male recipient (2.54, 1.16-5.75, p=0.02) were the only factors associated to relapse. Overall survival and relapse-free survival (RFS) after 10 years was 44%. RFS at 10 years in patients with no, grade I, II and III-IV acute GVHD was 40%, 65%, 52% and 0%, respectively (p=0.01). No effect of chronic GVHD on RFS was found.
Conclusion: No effect of acute or chronic GVHD on relapse was found. We found no evidence of any statistically significant graft-versus-MDS effect.

P1168

A beneficial effect of rapid clearance of bone marrow blasts prior to stem cell transplantation on prolonged survival of advanced MDS patients

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A retrospective analysis of influence of different clinical and laboratory parameters on disease outcome was performed in a cohort of 43 patients with advanced MDS(RAEB > 10% blasts + RAEB-T according to the FAB classification) who underwent allogeneic stem cell transplantation (SCT); 21 patients were transplanted with < 10% of bone marrow (BM) blasts after 1 or 2 courses of induction followed by 1 or 2 courses of consolidation chemotherapy (Group A), 22 patients were transplanted with > 10% BM blasts either treated with prior chemotherapy or transplanted up-front with intensified conditioning (Group B). Higher age (57.5v.s.35 years) and higher incidence of adverse karyotype were the only significant differences between Group B and Group A. Median survival of all transplanted patients was 35,5 months with a significant difference between Group A and B (57,5v.s.18,0 months, p=0.017). Estimated 3 year and 10 year survival were 53,5% and 41,9% respectively for all patients with and also differed significantly between Group A and B (71,4% and 57,1% for Group A and 36,4% and 27,3% for Group B). Complete remission (CR) rate was 44,2%, 18,6% patients relapsed (14,3% in Group A and 22,7% in Group B). No difference in survival was observed between patients with > 10% BM blasts transplanted either after chemotherapy or up-front. Univariate analysis using Kaplan-Meier curves and log-rank2 test revealed as significant variables affecting overall survival: achievement of CR (p=0.007), < 10% BM blasts prior SCT (p=0.011), SCT performed < 4 months after diagnosis (p=0.031) and absence of relapse (p=0.046). Independent variables for determining overall survival (studied by Cox regression multivariate analysis) were: SCT performed < 4

months after dg. (p=0.003), achievement of CR (p=0.01) and age < 50 years (p=0.044). None independent variable determining occurrence of relapse was found. Conclusions: combination chemotherapy leading to a rapid clearance of BM blasts below 10% followed by immediate SCT represented the best treatment option for younger patients with MDS with > 10% BM blasts. Patients transplanted with > 10% BM blasts at the time of SCT had significantly inferior outcome either transplanted after previous chemotherapy or up-front with intensified conditioning. In this subset of patients, a possible benefit of addition of hypomethylating agents to the treatment schedules prior SCT should be studied.

P1169

Allogeneic stem cell transplantation in patients with therapy-related MDS/AML following treatment with radioiodine

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Development of therapy-related MDS/AML (tMDS/tAML) after radioiodine treatment is a rare late effect and has been reported only sporadically. Patients with tMDS/tAML are often referred for consideration of allogeneic stem cell transplantation (allo-SCT). However, there a no data focussing specifically on allo-SCT as potentially curative option in patients (pts) with radioiodine-induced tMDS/tAML.

We here report on 10 pts (median age: 57 years, range: 19-66 years) who underwent allo-SCT for tMDS/tAML following radioiodine-therapy for benign (n=5) or malignant (n=4) thyroid diseases or carcinoid (n=1). Time from primary diagnosis to tMDS/tAML was 55 months (mo, range: 16-440 mo) and from tMDS/tAML to allo-SCT 6 mo (range: 3-22 mo).

At transplant, 6 pts had tAML (3 evolved from tMDS) and 4 pts had MDS (1 RCMD, 2 RAEB I, 1 RAEB II). IPSS was int-1/-2 in 3 pts and high in 1 pts. Six pts had intermediate-risk cytogenetics and 4 pts had high-risk cytogenetics. Seven pts had received induction therapy prior allo-SCT, of which 5 pts had primary induction failure. One pt suffered from 1st relapse, 1 pt was in CR1, while 3 pts were untreated. Thus, with regard to OS 8 pts had to be classified as being at high and 2 pts as moderate risk according to the EBMT risk model (Kröger et al., 2009).

Eight pts received a reduced intensity conditioning regimen, while standard dose conditioning was used in 2 pts. Three pts received grafts from related and 7 pts from unrelated donors. Stem cell source was PB in 8 pts, BM and CB in 1 pt, respectively. Following allo-SCT all pts engrafted. Median OS from allo-SCT is 12.5 mo (range 1-25 mo). Currently 3 pts (33%) are alive with a median follow-up of 12 mo (range 2-20 mo). Relapse occurred in 5 pts (50%) at a median of 119 days (40-232 days) after allo-SCT and all of them died. Two pts died as a consequence of TRM.

Comparing this group with a non-transplant group of another 23 pts with radioiodine-induced tMDS/tAML from our institution, allo-SCT did not translate into a better OS as calculated from diagnosis (allo-SCT: 21 mo, 95% CI 10 -33 mo vs 28 mo, 95% CI 2-55 mo, p= .405).

Concluding from our cohort, tMDS/tAML after radioiodine treatment is a considerable late effect associated with a poor prognosis even after allo-SCT despite a few patients with ongoing remission. This warrants further evaluation in a larger group of patients.

P1170

Dendritic cell, monocyte, B and NK lymphoid deficiency: a novel but potentially fatal haematological disorder curable with haematopoietic stem cell transplantation

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We describe a novel syndrome of dendritic cell (DC), monocyte, B and NK lymphoid (DCML) deficiency. The disorder presents in the 2nd to 4th decades of life and confers susceptibility to mycobacterial and viral infection, autoimmunity, pulmonary alveolar proteinosis (PAP) and acute leukaemia. In four cases, although the only significant abnormality on automated blood count was monocytopenia, we have shown that DC, B- and NK-cells are also depleted. These deficiencies are mirrored in tissues by absence of DC, with the exception of tissue macrophages and epidermal Langerhans cells which persist. DC deficiency is associated with disrupted immune regulation with reduction of circulating regulatory T cells in the context of massive elevation of serum fms-like tyrosine kinase receptor-3 (Flt3) ligand.

Despite normal bone marrow cytology, histology and cytogenetic analysis, flow cytometric analysis of the CD34+ compartment revealed complete absence of multi-lymphoid progenitors, and depletion of granulocyte macrophage progenitors, consistent with a primary bone marrow disorder. Our centre performed allogeneic Hematopoietic Stem Cell Transplantation (HSCT) for two patients, prior to the development of malignancy. The first entered transplant on treatment for chronic, disseminated BCG infection. The second was transplanted with respiratory failure secondary to PAP, necessitating non-invasive ventilation. Both received grafts from unrelated donors with fludarabine/busulphan-based conditioning and graft versus host disease (GvHD) prophylaxis with alemtuzumab, mycophenolate mofetil and/or ciclosporin. BCG infection resolved and respiratory symptoms improved shortly after engraftment in patients 1 and 2 respectively. At day 100 post transplant, patient 2 required oxygen only on exertion and chest radiography revealed marked improvement of lung pathology. Analysis of blood and skin at 3 months showed reconstitution of all PB and tissue DC subsets. Both patients remain alive and well with no chronic GvHD. Two subjects have not received HSCT: one died suddenly of H1N1 virus; the second has ongoing HPV infection, recurrent erythema nodosum and bacterial skin infections.

In summary, DCML deficiency is a primary haematological condition that may present as an isolated monocytopenia with diverse clinical symptoms. It is potentially fatal due to infection, PAP or transformation to acute leukaemia. HSCT represents an effective treatment where life-threatening complications occur.

P1171

Results of allogeniec haematopoietic stem cell transplantation in children and adolescents with myelodysplastic syndrome. Single-centre experience

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Background: Myelodisplastic syndrome (MDS) is a very rare hematological malignancy in childhood, its prognosis is very poor without allogeneic haematopoietic stem cell transplantation

(allo-HSCT). Allo-HSCT is the only potentially curative treatment for MDS in children and young adults.
The aim of this study: To evaluate efficacy of allo-HSCT in children and adolescents with myelodisplastic syndrome.
Patients and methods: Allo-HSCT received 21 patients (pts) with MDS; refractory anemia (n=1), refractory anemia with ringed sideroblasts (n=2), refractory anemia with excess of blasts (n=8), refractory cytopenia with multilineage dysplasia (n=1), juvenile myelomonocytic leukemia (n=1), acute myeloid leukemia (AML) secondary to MDS (n=8). HLA-matched sibling HSCT (n=2), matched unrelated HSCT (n=13) and alternative HSCT (haploidentical (n=5), cord blood (n=1)) were performed. Bone marrow (n=9), peripheral blood stem cells (PBSC) (n=8), bone marrow and PBSC (n=4) were used as source of hematopoietic stem cells. The median age at transplant was 9,6 years (range 1- 19). Male/female ratio was almost 1:1 (11/10). Among all pts 6 revealed monosomy 7 in cytogenetic examination. Reduced intensity conditioning regimen (RIC) received 14 pts, myeloablative conditioning (MAC) received 7 pts. MAC consisted Busulfan (Bu) 16 mg/kg + Cyclophosphamide 120 mg/kg. RIC included Fludarabine (Flu) 150 mg/m^2 + Melphalan (Mel) 140 mg/m^2 in 3 pts, Flu 150-180 mg/m^2 + Bu 8mg/kg in 10 pts, Flu 150 mg/m^2 + Mel 140 mg/m2+ Thiotepa 10 mg/kg in 1 pt.
Results: Engraftment was at day + 17 (range 12-32). Primary non engraftment was 33% (7 pts with excess of blasts or transformation in AML). The estimated 1-year overall survival (OS) was 43%. In patients with bone marrow HSCT, peripheral blood HSCT 1-year OS were 77% and 25%, respectively. 1-year OS of MAC allo-HSCT recipients and RIC allo-HSCT recipients were 50% and 40%, respectively. Patients with monosomy 7 had 1-year OS of 14% and patients with other cytogenetic abnormalities or with normal karyotype had 1-year OS of 57%.
Conclusions: Allo-HSCT is an effective treatment option for MDS in children and adolescents. The use of bone marrow compared to PBSC may be more beneficial in MDS patients. MAC allo-HSCT patients showed better 1-year OS in comparison with RIC allo-HSCT patients. Monosomy 7 positive patients have unfavorable prognosis.

P1172
Retrospective analysis of 5-azacitidine as induction therapy prior to haematopoietic stem cell transplantation
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Allogeneic haematopoietic stem cell transplantation(HSCT) is a potentially curative treatment for patients with high-risk myelodysplastic syndromes. While low disease burden (<5% blasts) at time of HSCT is associated with more favourable outcome, the most appropriate pre-HSCT induction remains to be determined. Novel agents such as 5-azacitidine (5-aza) may offer an advantage over intensive chemotherapy in terms of reduced toxicity while allowing time for donor identification. We performed a retrospective analysis of patients treated with 5-aza pre-HSCT with to assess transplant outcomes.
15 MDS/AML patients with median age of 57 yrs (range (R) 36-69) received pre-HSCT induction with 5-aza. Diagnosis included RAEBI (6), RAEBII (4), CMML (1), hypoplastic MDS (1), and RCMD (3). Cytogenetics at diagnosis were good/normal=3, intermediate=1, poor-risk=11. Median number of cycles of 5-aza was 7 (R:6-30). 5 patients had disease progression during 5-aza and required induction chemotherapy pre-HSCT. At HSCT all patients had <5% blasts, 7 patients had persistent cytogenetic disease. Donors included related (n=6), unrelated (n=7), cord (n=2). All patients received in-vivo T-cell depletion, with non-myeloablative (NMA) (n=7) and myeloablative (MA) (n=8) protocols.
All patients had neutrophil engraftment at a median of 12 days (R:9-19).Of 11 patients with available donor chimerism at day 30, all had CD15 full donor chimerism (FDC), and 8 had CD3 FDC.Median OS is 192 days (R:97-694) and median DFS 170 days (R:28-415). OS and DFS at 1yr is 40%±16% and 31%±12% respectively. At last follow-up, 5 patients remain alive (4 in remission) and 4 relapsed. Cause of death included GVHD/sepsis/CMV (4), relapse (2), pancreatic cancer (1), PTLD(1), respiratory failure (1) and unknown (1).Residual cytogenetic disease, diagnosis, donor type, and conditioning (MA vs RIC) had no influence on 1yr OS or DFS. A total of 67% (n=10) developed GVHD, 5(33%) with acute GVHD (3 with grade III-IV) and 7(47%) with chronic GVHD(all moderate to severe).
Our experience to date indicates 5-aza pre-HSCT is feasible with an acceptable incidence of relapse post-HSCT. Despite a median of 7 cycles of 5-aza pre-HSCT, the majority of patients had persistent cytogenetic disease at time of HSCT. Although 5-aza may provide an effective bridge to transplant it is notable that 33% of patients required additional pre-HSCT chemotherapy. Further prospective studies comparing 5-aza with intensive induction chemotherapy prior to HSCT are warranted.

P1173
Hypomethylating therapy with decitabine prior to allogenic BMT for MDS/AML patients
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Decitabine (Dacogen) (Dac) is a hypomethylating agent with activity in myelodysplastic syndromes (MDS). It is unknown whether treatment with the drug before allo SCT will increase the toxicity of the preparative regimen. Novel non-intensive treatment options in MDS patients planned for allografting, with the goal of down - standing disease and bringing time to transplantation are presently being developed.
We analysed the outcome of 9 MDS/AML patients (median age 39, range 18-54 years) were underwent an allogenic BMT from sibling (n=2), haplo (n=2) or unrelated donors (n=7) after prior therapy with Dac. At diagnosis 7 had high risk MDS by international prognostic scoring system, 2 – AML. 8 had a non-myeloablative, 1 ablative regimen.
The source of stem cells was bone marrow in 4, peripheral blood 2, both – 2.
The patients had received Dac for a median of 4,7 cycles (range 1-9) and a median duration of treatment of 6,3 month (range 1-13). Dac was well tolerated without severe complication.
Best response to Dac were: CR in 1(patient with AML), PR – 1 (patient with MDS), stabilization- 7 (6-with MDS, 1 with AML).
The median time between completion of Dac and transplantation was 7 months (range 2,5–14 month). At the time of transplantation 1 patient was in PR, 6 patients were in SD, 2 in PD. Mean CD 34+/kg cells count was 4,4 x106 cells (range 1,0–9,7).
8 patients engrafted and only one had primary engraftment failure (the patient with haploBMT). Mean time to neutrophil and platelets engraftment was 19 days (range 12–32) and 20 days (range 11–35) respectively . 3 patients (37%) developed acute GVHD (grade II), 2 chronic GVHD (22%). 5 patients were in CR within 100 days after transplantation. With a follow up of 19 months (range 1,4- 18,7 months) 5 patients are alive : 4 in CR, 1- after the second alloBMT, 4 died (1 –PD, 1- chronic GVHD, 1- thrombocytic thrombocytopenic purpura (TTP), 1- graft failure). TRM on day +100 was 23%. No TRM on day + 100 was in group of patients with HLA match donors.
Conclusion: The prior therapy with a hypomethylating agent Decitabine is feasible, allow to achive the complete remissions in same patients before alloBMT. This drug shows no unexpected toxicity, does not increase complications after transplantation and appears to be a part of pretransplant strategy in MDS/AML patients.

P1174

Outcome of children with severe and very severe acquired aplastic anaemia refractory to 2 courses of intensive immunosuppressive therapy

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Introduction: Although results of intensive immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine A (CsA) in children with severe and very severe aplastic anemia (SAA and vSAA) are comparable with those of HLA-identical stem cell transplantation (SCT), 20-30 % pts fail to recover hematopoiesis after 2 courses of IST. Here we report on the outcome of children with AA refractory to 2 courses of ATG.

Patients and methods: One hundred fifty three children (median age 10,9 mo) with SAA and vSAA were enrolled into the study of IST during 1999-2009. Hematological response was achieved after the first course of ATG (horse 133, rabbit 19, goat 1) in 83 (58,5%) pts, 11 pts died and 59 failed to respond. Of 59 non-responders 4 underwent SCT, 1 received alemtuzumab (Alem) and 53 got 2d course of ATG. Twenty four pts (45%) achieved response, while 29(55%) proved refractory. Results. Of 29 refractory pts 8 died before further therapy became possible, 11 underwent unrelated HLA-matched SCT and 10 survived. Ten pts received further attempts of immunosuppressive therapy: 5 received third ATG, 2 – Alem, 2-fludarabine (Flu) and 1 high dose of cyclophosphamide (Cy). Of the 5 recipients of 3d ATG 2 responded and alive, three did not respond and got SCT (1) or alem (2).

One of two pts who received Flu responded, as did two pts who received Alem. The recipient of Cy responded, but quickly relapsed and died. Overall survival was 10/11(90%) after SCT and 8/18(44%) in non-SCT group.($p<0,05$) Conclusions. Unrelated HLA-matched SCT is superior to further IST, although continuation of immunosupression with Alem or ATG may rescue some children with SAA and vSAA refractory to 2 courses of ATG+CsA.

P1175

Comparative survival between younger patients with poor risks and older patients with favourable factors in haematopoietic stem cell transplantation for adult aplastic aanemia

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Younger age is an important favorable prognostic factor to undergo HSCT in adult patients with aplastic anemia (AA), however other pre-transplant favorable factors can be more important than age factor. We speculated that the age factor could be overcome when other pre-transplant risk factors were favorable even in older patients. In this study we analyzed pre-transplant risk factors including the age factor and compared the survival between poor risk younger patients group and favorable older patients group in HSCT of adult AA. A total 225 adult AA patients who had undergone HSCT were enrolled in this study. The age at the time of HSCT in 57 patients were over 40 yrs (elderly group) and 168 patients were less than 40 years (younger group). We explored the pre-transplant prognostic factors on survival (Table 1). Age at alloHSCT ≤ 40 years (p=0.007), time from diagnosis to HSCT (p=0.008), sibling donor (p=0.002), HLA matching (p=0.019), ABO incompatibility (p=0.055), prior PC transfusion ≤ 86 U vs >86 U (p=0.063) were favorable risk factors on survival. Mutivariate analysis revealed age at alloHSCT >40 years (HR 0.403, 95% CI 0.227-0.715; p=0.002), time from Dx to alloHSCT >6 months (HR 0.464, 95% CI 0.247-0.872; p=0.017) and unrelated donor (HR 0.524, 95% CI 0.294-0.933; p=0.028) were significantly poor prognostic factors for survival. Prognostic factors among patients over 40 yr were analyzed (Table 2). Only age over 50 yr were a significantly poor risk factor on survival (HR 0.307, 95% CI 0.126-0.748; p=0.009). We compared the survival between younger group with poor risk factor and older group with good prognostic group. There were no survival differences between younger group who had received from sibling donor and older group who had received from unrelated donor (p=0.850). Also no difference was found between younger group who was time from Dx to SCT < 6M and older group who was time from Dx to SCT > 6M (p=0.905).

These findings suggested that good prognostic factors (sibling donor and time from Dx to SCT < 6M) could overcome the age factor especially under 50 years old patients. In conclusion, when the patient's age is under 50 years and has a sibling donor, alloSCT should not be delayed.

Table 1. Univariate analysis on survival

Factor	n	5-year survival rate (%)	p-value
Female vs. male	117 vs. 108	74.0 vs. 67.7	0.640
No prior IST vs. prior IST	122 vs. 103	77.4 vs. 61.9	0.005
Age at alloHSCT ≤40 years vs. >40 years	168 vs. 57	76.6 vs. 55.9	0.007
Time from Dx to alloHSCT ≤6 months vs. >6 months	107 vs. 118	78.7 vs. 62.8	0.008
Sibling donor vs. Others	162 vs. 63	75.6 vs. 56.2	0.002
ABO compatible vs. incompatible	95 vs. 110	75.9 vs. 59.3	0.055
HLA full match vs. mismatch	185 vs. 40	73.1 vs. 55.9	0.019
Others vs. female donor-to-male recipient	186 vs. 39	71.8 vs. 63.1	0.499
ECOG performance status at alloHSCT; >1 vs. ≤1	33 vs. 192	71.8 vs. 70.0	0.948
Prior PRC transfusion ≤12 U vs. >12 U	160 vs. 65	71.9 vs. 65.9	0.396
Prior PC transfusion ≤86 U vs. >86 U	149 vs. 76	75.3 vs. 58.7	0.063
Conditioning without vs. with TBI	24 vs. 201	70.9 vs. 65.0	0.525
Conditioning with vs. without ATG/ALG	173 vs. 52	71.6 vs. 65.5	0.628
Cy-ATG/ALG conditioning vs. other	170 vs. 55	71.6 vs. 65.6	0.585
BM as a stem cell source vs. Others	179 vs. 46	72.9 vs. 51.6	0.224
Infused CD34 infusion >3 vs. ≤3 ($\times 10^6$/kg)	144 vs. 81	73.3 vs. 65.6	0.305

Table 2. Prognostic factors on survival in patients over 40 years

Factor	n	Univariate analysis		Multivariate analysis		
		5-year survival rate (%)	p-value	HR	95% CI	p-value
Female vs. male	29 vs. 28	51.4 vs. 59.8	0.469	-	-	-
No prior IST vs. prior IST	32 vs. 25	62.0 vs. 47.2	0.123	0.664	0.269-1.642	0.376
Age at alloHSCT >40 and ≤50 years vs. >50 years	41 vs. 16	76.6 vs. 55.9	0.007	0.307	0.126-0.748	0.009
Time from Dx to alloHSCT ≤6 months vs. >6 months	30 vs. 27	62.2 vs. 48.3	0.129	0.563	0.208-1.521	0.257
Sibling donor vs. Others	43 vs. 14	58.5 vs. 46.8	0.405	-	-	-
ABO compatible vs. incompatible	29 vs. 25	56.8 vs. 54.0	0.830	-	-	-
HLA full match vs. mismatch	49 vs. 8	60.0 vs. 22.5	0.023	0.472	0.156-1.426	0.183
Others vs. female donor-to-male recipient	43 vs. 14	58.2 vs. 49.7	0.890	-	-	-
ECOG performance status at alloHSCT; ≤1 vs. >1	46 vs. 11	52.1 vs. 70.1	0.297	-	-	-
Prior PRC transfusion ≤12 U vs. >12 U	33 vs. 24	56.5 vs. 53.3	0.957	-	-	-
Prior PC transfusion ≤86 U vs. >86 U	28 vs. 29	55.2 vs. 54.6	0.933	-	-	-
Conditioning without vs. with TBI	50 vs. 6	54.1 vs. 66.7	0.430	-	-	-
Conditioning with vs. without ATG/ALG	41 vs. 16	57.4 vs. 53.0	0.683	-	-	-
Cy-ATG/ALG conditioning vs. other	41 vs. 16	57.4 vs. 53.0	0.683	-	-	-
BM as a stem cell source vs. Others	42 vs. 13	61.4 vs. 33.4	0.950	-	-	-
Infused CD34 infusion >3 vs. ≤3 ($\times 10^6$/kg)	34 vs. 23	56.2 vs. 55.3	0.740	-	-	-

P1176

Increasing the dose of rabbit-ATG does not lead to a higher response rate in the first-line treatment of severe aplastic anaemia

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Immunosuppressive treatment (IST) with horse anti-thymocyte globulin (h-ATG) and cyclosporine has been used for patients with aplastic anemia (AA) who are not candidate for allogeneic stem cell transplantation (alloSCT). H-ATG became unavailable in Europe and since 2008, rabbit ATG (r-ATG) is used. A prospective randomized trial by NIH showed r-ATG to be inferior to h-ATG (hematological response (HR) rates at 6 months 35% versus 69%, respectively). Moreover, patients treated with r-ATG showed a lower overall survival, mainly due to high mortality after alloSCT as 2nd or 3rd line treatment. In the absence of dose finding studies, r-ATG 3.75 mg/kg/day for 5 days is advocated. Data from a Spanish national study suggested that the efficacy of r-ATG could be improved by increasing the dose. However, high dose r-ATG has been associated with EBV-related disease. We compared the response rate of h-ATG and high dosed r-ATG (5 mg/kg/day for 5 days) in 22 transfusion-dependent acquired AA patients treated between 2000 and 2010 in our hospital.

Until 2008 13 patients (10 males, median age 31) received h-ATG. From 2008 onwards, 9 patients (6 male, median age 33) were treated with Thymoglobulin (r-ATG) 5 mg/kg/day for 5 days. Cyclosporine was started in all patients at day 1 with target plasma concentration of 150-250 ug/L). Complete HR (CHR) was defined as normalization of blood counts. Partial HR was defined as transfusion independency without CHR. After 6 months, overall HR was observed in 9 (69%) patients treated with h-ATG and in 3 (33%) with r-ATG. 3/13 patients treated with h-ATG and 0/9 patients treated with r-ATG developed a CHR. No EBV reactivations occurred after r-ATG. Of the 6 non-responding r-ATG patients, 5 underwent alloSCT as 2nd line treatment. One patient died of the complications of acute GVHD, whereas the other 4 transplanted patients are in CR without GVHD (median follow-up of surviving patients after SCT 495 days, range 280-693).

In conclusion, treatment with r-ATG 5 mg/kg/day for 5 days results in a similar response rate of 33% as recently published for lower dosed r-ATG, whereas patients treated with h-ATG had a response rate of 69%, comparable to published results. These results illustrate the need to re-introduce h-ATG in Europe for optimal treatment of AA patients.

P1177

GvHD prophylaxis with or without MTX in patients receiving sibling transplants for SAA. Impact on graft failure

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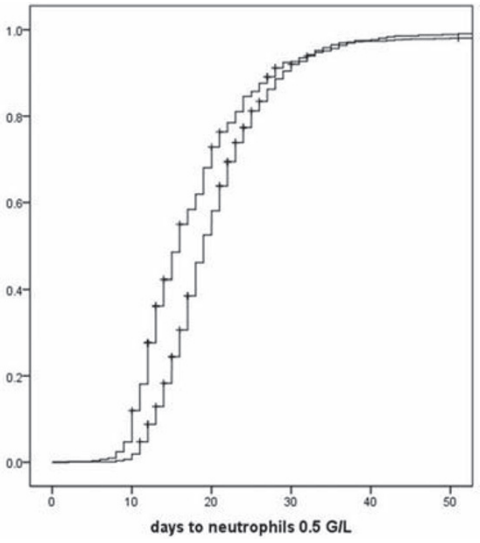
This is a study on 2392 patients receiving sibling transplants 1980-2009 for aplastic anemia using either marrow (1972, 82%) or peripheral blood (420, 18%), thus excluding recipients of cord blood and excluding T-cell depleted transplants in order to analyze the impact of added methotrexate (MTX) (1504, 63%) as compared to GvHD prophylaxis with cyclosporine (CSA) alone (888, 37%) on the risks of graft failure. In aplastic anemia the rate of graft failure remains higher than in other diseases and it was hypothesized that MTX might be exerting a posttransplant immunosuppressive effect reducing graft failure risks. Secondary outcomes are the incidence of GvHD and survival in the 2 cohorts.

There were significant differences among groups. Median age was 17 (<1-70) in patients without MTX and 21 (<1-67) in patients with MTX (p<0.0001). The median year of transplantation was 1993 for patients without and 1999 for patients with MTX (p<0.0001). Median delay from diagnosis to transplantation was 131 days without and 117 days with MTX (p=0.03).In patients transplanted with peripheral blood 71% had MTX as compared to 61% with marrow (p<0.0001).

Median time to neutrophil engraftment was 16 (15-17) days without and 19 (18-20) days with MTX (p<0.0001) (Figure). There were no significant differences in the frequencies of primary nonengraftment 2.2% without and 3.5% with MTX or late graft failure 0.9% without and 1.4% with MTX. The probability of grade II-IV acute GvHD by d100 was 22 + 3% without and 12 + 2 (p<0.0001) with MTX in univariate analysis and survival at 5 years was 73 + 3% without and 78+ 2% (p< 0.008) with MTX. This survival benefit, however disappeared in multivariate analysis after adjustment for patient age, year of transplant, interval from diagnosis to transplant and stem cell source.

Conclusions: GvHD prophylaxis with MTX delays engraftment by approximately 3 days. There is no evidence that GvHD prophylaxis with MTX decreases the rate of early or late graft failure in patients with SAA receiving identical sibling HSCT. Given the evidence of effectivenes of MTX for GvHD prophylaxis in randomized trials the use of MTX is clearly recommended.



P1178

Biological and functional characterization of ex vivo expanded BM-derived MSCs from Fanconi anaemia patients

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Objective: Fanconi Anemia (FA) is a genetically and phenotypically heterogeneous, autosomal recessive disorder characterized by various degree of pancytopenia, congenital malformations and predisposition to develop malignancies. Alterations in the hematopoietic microenvironment of FA patients have been reported, but little is known on the components of their marrow stroma, in particular on mesenchymal stromal cells (MSCs). In the present study, we functionally and genetically characterized FA-derived MSCs (FA-MSCs).

Methods: MSCs were isolated and expanded ex vivo from bone marrow (BM) of 10 FA patients (mean age 7, range 2-10 years). Clonogenic efficiency (CFU-F), proliferative capacity

(cumulative cell count), morphology, immunophenotype (flowcytometry), differentiation potential and ability to suppress in vitro proliferation of lymphocytes to phytohemagglutinin (PHA) were analysed. Genetic stability of MSCs was studied by both array-comparative genomic hybridization (array-CGH) and conventional karyotype. Results were compared with those obtained from BM-MSCs of 9 age-matched healthy donors (HD).
Results: CFU-F frequency was significantly lower (p=0.023) in FA-MSCs compared to HD-MSCs (mean±SD:3±2 and 5±1, respectively), while proliferative capacity and surface immunological markers did not differ. FA-MSCs displayed the typical spindle-shaped morphology and were able to differentiate into both adipocytes and osteoblasts. Both in the autologous (FA-MSCs/FA-PBMCs) and allogeneic (FA-MSCs/HD-PBMCs) settings, FA-MSCs inhibited in vitro proliferation of lymphocytes to PHA by up to 39% and 61%, respectively; whereas in the autologous (HD-MSCs/HD-PBMCs) and allogeneic (HD-MSCs/FA-PBMCs) settings, HD-MSCs reduced PBMC proliferation by up to 95% and 90%, respectively. Array-CGH analysis carried out in all ex vivo expanded FA-MSCs did not exhibit chromosomal abnormalities, while conventional karyotype showed chromosomal breakages (not detected in HD-MSCs) in 3/3 patients evaluated. Chromosomal breakages were different in every metaphase and they were not related to culture passages.
Conclusion: Our results indicate that, as compared to HD-MSCs, FA-MSCs show an impaired capacity to suppress in vitro T cell proliferation in response to mitogenic stimuli and display chromosomal fragility, characteristic of FA-hematopoietic cells. Whether these functional and genetic defects are involved in the pathophysiology of the disease remain to be investigated.

P1179
The Italian Registry of paediatric acquired aplastic anaemia: a retrospective survey
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Objectives: Aplastic anemia (AA) is a rare acquired bone marrow failure disorder (2 cases/million/year) and data collection on epidemiology and treatment from national registries is needed. Immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporin (CyA) is standard treatment when lacking a family donor. Addition of granulocyte colony stimulating factor (G-CSF) may shorter neutrophil recovery but its prolonged use can increase risk of clonal disease. Results of hematopoietic stem cells transplantation (HSCT) from a matched unrelated donor (MUD) in AA have improved by using a reduced intensity conditioning regimen. Recently EBMT and AIEOP guidelines suggest MUD HSCT in young patients with severe AA who do not respond to first treatment (I-IST). Aim of the study is to report on compliance to EBMT and AIEOP guidelines by analyzing data from the newly implemented Italian Registry of pediatric AA (by a modified EBMT-PROmisE database).
Methods. The Registry started patient enrollment in 2010 including children (0-18 years) with AA consecutively diagnosed at AIEOP and other non pediatric centres and treated with IST (prospective Registry from 2009, now in progress with a total of 19 participating centres; retrospective survey performed among AIEOP centres from 1999 to 2009, with total of 120 patients).
Results: We report on preliminary data analysis on 75/120 patients (1999-2009): 50 males, 25 females, median age 9,2 years. All were treated with IST according to EBMT/AIEOP protocol and all but 18 received G-CSF. Response to I-IST was achieved in 35/75 (46,6%) by day +180; among the remaining 40 patients, 21 (52,5%) received II-IST (nine (42,8%) are alive and responders; 12 (57,2%) non-responders underwent HSCT and 10 of them are alive); 19 non responders to I-IST (47,5%) received HSCT, 16 are alive (84,2%) and 3 died for HSCT related complications. Clonal evolution occurred in 3 patients. Relapse occurred in 2 out of 35 responders (5.7%):1 received HSCT and 1 died for infection. At last follow up 42 patients are alive and in remission post-IST (20 off treatment and 22 on CSA), one died for infection after relapse, 27 are alive post HSCT, and 5 died after HSCT.
Conclusion: In Italy AA children were treated with IST with full compliance to AIEOP/EBMT guidelines; HSCT was performed in almost half of non responders to I-IST, in alternative to II-IST. The Registry will be in the future a useful tool for prospective studies.

P1180
Allo-HCT from MUD/MRD as a curative treatment in paroxysmal nocturnal haemoglobinuria
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Allo-HCT is the only curative treatment for PNH, although outcomes presented in the past were controversial. Thus it is important to re-evaluate results of allo-HCT in PNH. We report 19 allo-HCTs: 17 from MUD and 2 from MRD performed for PNH in 2004-2010. Median age of recipients was 28 years (range 20-51) and donors 33(19-53), median time from diagnosis to allo-HCT was 16(2-97) months. Median size of PNH clone was 90% granulocytes (4%-98%). Indication for allo-HCT was aplastic/hypoplastic bone marrow (10 pts), MDS (1 pt), severe course of PNH with hemolytic crises (4 pts) and transfusion-dependency (9 pts). Additional risk factors were Budd-Chiari syndrome and hepatosplenomegaly (1 pt), history of renal insufficiency requiring hemodialyses (2 pts) and chronic hepatitis B (1 pt). The preparative regimen consisted of treosulfan 3x14 g/m2 plus fludarabine 5x30 mg/m2 (17 pts) or treosulfan 2x10 g/m2 plus cyclophosphamide 4x40 mg/kg (2 pts). Standard GVHD prophylaxis consisted of cyclosporine-A, methotrexate and pre-transplant ATG or thymoglobulin in MUD-HCT. Source of cells was bone marrow (9 pts) or peripheral blood (10 pts) with median 2.9 or 11.0 x10(8)NC/kg, 2.4 or 7.3 x10(6)CD34+cells/kg, 26.2 or 245.9 x10(6)CD3+cells/kg, respectively. Myeloablation was complete in all pts with median 9 days (6-13) of absolute agranulocytosis <0.1 G/l. Median number of transfused RBC and platelets units was 7(0-12) and 6(3-15). All pts engrafted, median counts of granulocytes 1.0 G/l, platelets 50 G/l and Hb 10 g/dl were achieved on days 17(13-39), 17(9-39) and 30(16-50). Acute GVHD grade I,II and III was present in 8, 5 and 1 pt, limited chronic GVHD in 6 pts. LDH decreased by 78%(5%-91%) in first 30 days indicating disappearance of hemolysis. 100% donor chimerism was achieved in all pts. 1 previously hemodialysed pt died on day +102 in a consequence of nephrotoxicity complicating adenoviral/CMV hemorrhagic cystitis. Complications in survivors were FUO (5 pts), CMV reactivation (3), VOD (1), neurotoxicity (1), venal thrombosis (1), hemorrhagic cystitis (1) and mucositis (3). 18 pts (94.7%) are alive 16 months (2-61) post-transplant and are doing well without treatment. Complete disappearance of PNH clone was confirmed by flow cytometry in all surviving pts. Allo-HCT with treosulfan-based conditioning is effective and well tolerated curative therapy in PNH.

P1181

Long-term follow-up of haematopoietic stem cell transplantation in patients with severe aplastic anaemia after conditioning with cyclophosphamide plus antithymocyte globulin

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Background: The literature on the long-term follow-up (FU) of patients (pts) with SAA who received an hematopoietic stem cell transplantation (HSCT) from an HLA-identical sibling donor after conditioning with cyclophosphamide (Cy) and Antithymocyte globulin (ATG) is still limited.
Methods: We retrospectively analyzed 61 pts with SAA who received an HSCT from an HLA-identical donor from June 1991 to February 2010, following CY (200mg/Kg) and ATG (2.5 mg/kg/day x 5 days). Graft-versus-host disease (GvHD) prophylaxis consisted in CSA and MTX.
Results: All pts had acquired SAA (49 idiopathic, 80.3%; 7 post-hepatitis, 11.5% and 5 PNH, 8.2%). The median age was 29.2 years (yrs)[range: 3.7- 42.8], 41 being adults. Median duration of the disease before HSCT was 3.1 months (mths) [1–140]. All but 2 pts received bone marrow (BM) as source of stem cells. All but 1 donor were genotypically HLA-identical siblings. Median infused cell dose was 2.9×107 TNC /kg [0.04- 21.6] and 4×107 CD3/kg [0.9-22.7]. All but 2 pts engrafted. The median time for neutrophils and platelets recovery (>500/mm3 and >20 000/mm^3) was 23 days [19–99] and 21 days [10-177], respectively. Cumulative incidence (CI) of acute grade II-IV GvHD was 23% (95%CI, 13-34). 18 developed chronic GvHD (CI:33%, 95% CI, 20-45). In multivariate analysis, for pts who received BM, higher number of infused CD3 cells (continuous variable) was associated with an increased risk of developing chronic GvHD (p=0.014). With a median FU of 65 mths [3- 582], the estimated probability of 6-yrs overall survival was 86%. The main cause of death was fungal infection (3 out of 6). 5 deaths occurred within the first 2 yrs after HSCT. The CI of secondary malignancies at 96 mths was 13% (95%CI, 4-26) (3 basal cell carcinoma, 1 EBV-associated Hodgkin lymphoma, 2 uterin cervix carcinoma). 10 pts presented an avascular necrosis during FU (CI of 21% at 72 mths (95%CI, 10-35). 11 pts presented endocrine dysfunctions (3 diabetes, 3 thyroid problems and 4 dyslipidemia) (CI of 19% at 72 mths (95% CI, 9-31) and 5 pts presented cardiovascular complications (4 high blood pressure and 1 myocardial infarction) (CI of 10% at 72 mths (95%CI, 3-22).
Conclusions: CY and ATG is an effective conditioning regimen for pts with SAA with low treatment-related mortality. However, pts are exposed to long-term complications (mainly avascular necrosis and secondary malignancies), as well as chronic GvHD, those who received a higher number of CD3 cells.

P1182

Allogeneic haematopoietic cell transplantation in patients with severe aplastic anaemia in Denmark. Marked improvement in long-term survival after year 2000 with both related and unrelated donors

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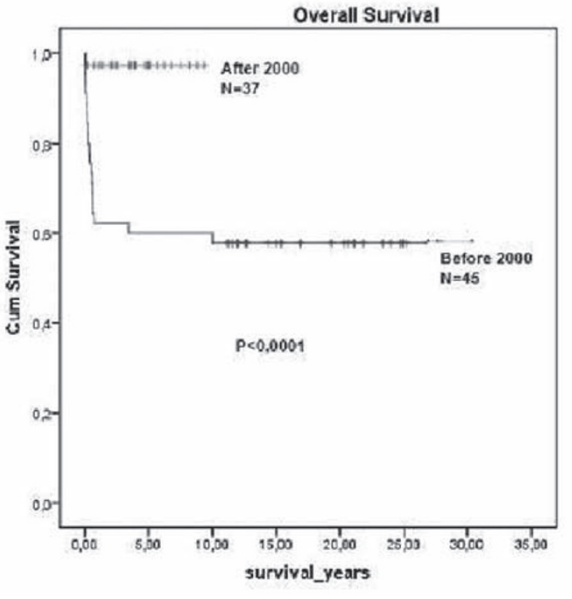
Treatment of severe aplastic anemia (SAA) is based on immunosuppressive therapy (IST) and hematopoietic cell transplantation (HCT). Several studies have shown an improved survival in patients treated with HCT in recent years, most pronounced in transplants with related donors. We retrospectively analyzed the outcome for all patients treated with HCT for SAA in a single institution in Denmark between 1977 and 2010.
Charts from 82 consecutive patients transplanted at our institution were analyzed. The median age at HCT was 16 years (0-61), 43 (52 %) were male and 39 (48 %) female. Donor source was HLA identical sibling in 46 (56 %), syngen in 2 (2 %), related HLA mismatched in 4 (5 %), HLA matched unrelated in 21 (26 %), and HLA mismatched unrelated in 6 (7 %). Except for a few early transplants, the standard regimen for patients with a HLA identical sibling was Cyclophosphamide (CTX) 200 mg and ATG. For patients with an HLA matched unrelated donor, the conditioning was CTX 200 mg, total body irradiation (TBI), and ATG. After the year 2000, total body irradiation (TBI) dose was reduced from 1200 cGy to 200 cGy. Forty-five (55 %) patients were transplanted between year 1977 and 2000, and 37 (45 %) were transplanted after year 2000. Other than conditioning regimen, there were no differences between the two cohorts (Table 1). As graft source, 78 (95%) patients received bone marrow, 4 (5%) received peripheral stem cells.
The overall survival (OS) was 76 % with an median observation time of 7,8 years (0,14-30,3). Overall survival in patients transplanted before 2000 was 58 %, whereas survival after 2000 increased to 97 % (P<0.0001), median observation times were 20,5 years (11,2-30,3) and 3,8 years (0,14-9,9), respectively (Fig 1). Among transplants with unrelated donors, the OS before and after year 2000 was 46 %, and 100 %, respectively (median observation time 12,6 years (11,9-21,2) and 3,5 years (0,14-7,5), p<0,005. Donor selection (related/unrelated), nucleated cell dose infused, duration of disease before HCT and age did not influence on survival.

Table 1. Characteristics in patients treated before or after 2000

	Before 2000	After 2000	P
N	45	37	
Median age in years (range)	12,9 (0,6-43)	20,1 (0,4-61)	0,13
Gender male n (%)	27 (60%)	16 (43%)	0,13
Disease duration at HCT			
≤ 12 months	29 (65%)	18 (49%)	0,15
>12 months	12 (26%)	15 (40%)	0,18
N/A	4 (9%)	4 (11%)	0,77
HLA match			
Matched sibling	27 (60%)	20 (54%)	0,59
Syngeneic	1 (2%)	1 (3%)	0,89
Mismatched related	4 (9%)	0 (0%)	0,06
Matched unrelated	10 (22%)	12 (32%)	0,30
Mismatched unrelated	3 (7%)	4 (11%)	0,50
Gender matching (recipient/donor)			
Male/Male	15 (33%)	9 (24%)	0,37
Male/Female	12 (27%)	7 (19%)	0,41
Female/Female	11 (25%)	11 (30%)	0,60
Female/Male	6 (13%)	10 (27%)	0,12
N/A	1 (2%)	0 (0%)	0,36
Graft source			
BM vs PBSC	44 vs 1	34 vs 3	0,22



In conclusion, we have observed a marked improvement in survival among SAA patients receiving an allogeneic HCT in Denmark since 2000. The improved survival also holds true for transplantations with unrelated donors. These encouraging results should be kept in mind when evaluating SAA patients for transplantation with an unrelated donor.

P1183

Better outcome with fludarabine-based conditioning in Fanconi anaemia

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Fanconi anemi (FA) is a rare genetic disorder characterized by congenital malformations, progressive bone marrow failure and predisposition to malignancy. Hematopoietic stem cell transplantation (HSCT) is the only treatment modality with the potential of correcting the hematological manifestations of FA. Because of sensitivity to DNA cross-linking agents and tendency to malignancy less toxic conditioning protocol should be choosen. We report here our experience with 26 FA patients who underwent HSCT with irradiation or non-irradiation based preparative regimens.
Between July 2000 and September 2010, 26 children (18 male and 8 female) with FA underwent HSCT. Donors were HLA matched siblings in 11, HLA matched parents in 5 and HLA matched unrelated donor in 10 patients. The median age at transplantation was 10 (range 3 to 17 years). In 21 patients peripheral blood, in 4 patients bone marrow and in one patient cord blood were used as stem cell source. Two different conditoning regimens were used; regimen A consisted of antithymocyte globulin (ATG) 10 to 20 mg/kg/d for 3 days (ds), cyclophosphamide (CY) 5 mg/kg/d for 4 ds, and thoracoabdominal irradiation (TAI, total 5 Gy) (n:6); regimen B consisted fludarabine (FLU) 30 mg/m^2/d for 4 ds, CY 10/mg/d for 4 ds and ATG 20 mg/kg/d for 3 ds (n:20). The first 6 patients received regimen A. Patients who underwent unrelated HSCT received FLU 30 mg/m^2/d for 5 ds, ATG 30 mg/kg/d for 3 ds and CY 10 mg/kg/d for 4 ds. Cyclosporin A (CsA) was given in related and sibling transplant and mycophenolate mofenil or prednisolon was added to CsA for unrelated donors for GVHD prophylaxis.
Engraftment was sustained in 25/26 patients. Five of 6 patients in regimen A and 6 out of 20 patients in regimen B experienced GVHD. Post-transplant AML developed in one patient in regimen A group. There is no secondary malignancy in regimen B group. Two patients needed a second transplant and died afterwards. Two out of 6 patients in regimen A are alive with full donor chimerism whereas 16 of 20 patients who received FLU based conditioning are well.
In conclusion, the conditioning protocol for FA patients had been changed in our center in the course of time and our experience also showed that FLU based conditioning regimen is well tolerated with lower GVHD and better survival in HSCT from related or unrelated donors. However, it needs more experience and longer follow up duration.

P1184

Pharmakokinetics of pegylated filgrastim in children with severe and very severe aplastic anemia after immunosuppressive therapy

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Introduction: Although addition of granulocyte colony-stimulating factor G-CSF doesn't impact long term results of immunosupressive therapy(IST) of severe and very severe aplastic anemia (SAA and vSAA) in children, G-CSF is still used widely with a goal of increasing granulocyte count and protecting pts against early life-threatening infections while waiting for recovery of stable hematopoiesis. Due to short half-life of conventional G-CSFs they have to be administered daily for a prolonged period of time what cause significant inconveniences to children. In neutropenic pts pegfilgrastim has greatly extended half-life due to reduced neutrophil-mediated clearance and thus warrants to be studied in children with AA. We hypothesized that pharmacokinetics of pegfilgrastim in AA might be different compared with patients with solid tumors, who typically recover from neutropenia with supernormal granulocyte counts and rapidly clear the excess of pegfilgrastim.
Patients and methods. Twenty one pts (9 M, 12 F) aged 4-17 (med 10,8) yrs with SAA (2pts) and vSAA(19 pts) lacking HLA-identical donor entered into the study. Nineteen pts received horse antithymocyte globulin(ATG) and 2 pts received alemtuzumab (Alem), followed by cyclosporine A. Pegfilgrastim 100 mcg/kg bw (max dose 6 mg) was administered sc biweekly starting from day 1 after the end of ATG or Alem for a total of 3 doses in first 11 pts, while in the last 10 pts interval between 2d and 3d dose of pegfilgrastim was 3wks. Blood counts with manual count of granulocytes was done daily. Serum levels of filgrastim were accessed daily in duplicate by ELISA throughout the study.
Results: Pegfilgrastim was well tolerated with no immediate side effects. Of 21 pts enrolled 13 showed rise of granulocytes >500/mcl, 6 failed to respond and in two granulocytes at least doubled over initial counts and ranged from 300 to 490/mcl. On days 14 after 1st and 2nd dose of pegfilgrastim filgrastim serum levels were 3004±6486 (range 82-30000, med 742) and 4508±8782, med 1203) ng/ml respectively - all above pharmacologically active levels. Moreover, in pts with the interval of 3 wks between 2d and 3d dose of pegfilgrastim through levels were 887 ±1653 med 326 ng/ml, which also exceed values, considered as therapeutically active. Conclusions. Pegfilgrastim in children with SAA and vSAA has a slow clearance and its therapeutic levels are maintained for at least 2-3 weeks. Further studies of its efficacy are warranted.

P1185

Allogeneic stem cell transplantation as treatment of marrow failure syndromes:a single-centre experience

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Objectives: To analyze the results of stem cell transplantation (SCT) as treatment of marrow failure syndromes in terms of survival and complications and to investigate the influence of chimerism status on graft rejection and graft versus host disease (GvHD) incidence.
Patients and methods: Fifty-six consecutive patients received an allogeneic SCT at our institution for treatment of marrow failure (Jan1995-Dec2009). Diagnostic were the following: Severe aplastic anaemia (n=44), Paroxysmal nocturnal hemoglobinuria (PNH) (n=4), Fanconi anaemia (n=4) and Blackfan-Diamond Syndrome (n=4). Four patients received a second SCT because of late graft rejection (n=3) or HPN relapse (n=1). Thus, 60 patients were available for analysis.
The median age at transplantation was 21 years (7-54). Fifteen per cent of the patients were ≤18 years old. 56.7% were men. The source of stem cell was: bone marrow (n=53), peripheral blood (n=6) and umbilical cord blood (n=1). 81.7% of the patients received a SCT from an HLA identical sibling donor and 11 from a matched unrelated donor (MUD). The conditioning regimen varied according to pathology and date of transplant. 53.3% received ATG as part of conditioning, 13.3% total body irradiation (5-6Gy) and 30% total nodal irradiation. As GvHD

prophylaxis all patients have received cyclosporine based regimen, in 66.7% of cases combined with methotrexate.
Results: The median follow-up was 7 years (14 days-19 years). aGvHD II-IV incidence was 20% and 16.6% for cGvHD, suffering 4 patients a severe form. Overall survival was 83.3% at ten years. However, it has improved in the last decade: 100% (HLA identical sibling) and 75% (MUD) (p=0.021). The transplant related mortality (TRM) of the entire series was 16.6% and 7% in the last decade. It is significantly higher in MUD (p=0.002). No patient has developed myelodysplastic syndrome or PNH clone after SCT.
Chimerism status post-SCT has been studied: VNTR loci (1995-2004) and real time quantitative PCR (2004-2009).24 out of 34 patients reached full donor chimerism status in a median time of 2.5 months post-SCT (range:15 days-4.3 years). Stable mixed chimerism was observed in 10 patients without evidence of relapse or graft failure.
Conclusions: The overall survival of allo-SCT for treatment of marrow failure syndromes has improved in the last years, being 83.3% in our global series and 93% in the last decade. The mixed chimerism status post-SCT is frequent and it does not predict relapse or graft rejection.

P1186
Haematopoetic stem cell transplantation from identical sibling and matched unrelated donor for severe aplastic anaemia at University Hospital Bratislava
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Objectives: Immunosuppresive treatment (IS) and hematopoetic stem cell transplantation (HSCT) are both effective therapies for severe aplastic anemia (SAA). The initial therapy is primarily driven by HLA identical sibling availability and patient's age. We evaluated our patients' outcomes after allogeneic HSCT from HLA-identical siblings and HLA-matched unrelated donors.
Methods: From August 1991 until February 2010 23 patients with SAA underwent HSCT at our transplantation unit (median age 23 years, range 18-23 years). 18/23 pacients underwent allogeneic transplantation from identical sibling, 1 patient from identical twin (syngeneic transplantation). Conditioning regimen included cyclophosphamide (CY 50 mg/kg 4 days) (9 patients), CY + antithymocyte globulin (ATG, Thymoglobulin 30 mg/kg 3 days) (10 patients). 4 patients underwent allogeneic HSCT from matched unrelated donor after initial IS failure (1-2 cycles), conditioning regimen included CY+ATG+fludarabine (CY 300 mg/m², fludarabin 30 mg/m², ATG 1,5 vial/10 kg, 4 days). Source of stem cells was dominantly bone marrow (20 patients). Graft versus host disease (GvHD) prophylaxy was provided by combination of methotrexate (MTX) + cyclosporin A (CsA), in 4 patients CsA + mycophenolate mofetil, methylprednisolone in 1 patient, 1 patient (after syngeneic HSCT) did not get IS.
Results: To date 31/05/2010, 18/23 patients are alive (78%), 13/19 (68%) after HSCT from related sibling and all 4 patients after matched unrelated HSCT. Engraftment i.e. neutrophil increase above 0.5 G/l was achieved in 12-40 days after HSCT (median 19 days), acute GvHD (gr.I-II) developed in 4 patients. 3/21 patients who rejected were retrasplanted, 1 patient after 2nd HSCT is alive.
Conclusion: Allogeneic HSCT from an HLA-matched sibling and unrelated donor provides curative therapy for SAA, results have improved over time.

Autoimmune diseases

P1187
Haematopoietic stem cell transplantation in paediatric patients with refractory autoimmune cytopenia: a retrospective analysis from the EBMT registry
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The analysis describes the outcome of pediatric patients receiving an haematopoietic stem cell transplantation (HSCT) to treat severe refractory autoimmune cytopenias.The registry of the EBMT contains data on 24 patients (14 males, 10 females) receiving 26 transplants. Patients had Evans's syndrome (10), autoimmune haemolytic anemia (6), immune thrombocytopenia (5) and autoimmune lymphoproliferative syndrome (3). The median age at diagnosis was 4 yrs. (range 0.3-16) with a median age at transplant of 7.1 yrs (1.5-17). All patients failed multiple second and third line immunesuppressive treatments with a median disease duration of 41 months (1-180 months).
Transplant were autologous for 7 and allogeneic for 19 patients, 7 of these transplanted from an HLA identical donor, 9 from a matched unrelated donor and 3 from a family mismatched donor. One patient received 2 autologous transplant while another patient received an allogeneic transplant after the first autologous. The stem cell source in the allo group was bone marrow in 11, mobilized PBSC in 6 and cord blood in 3 while in the autologous group always mobilized PBSC. The conditioning regimen used were heterogenous.
7 patients died of treatment related mortality, 6 in the allo and 1 in the auto group with a total TRM of 26%. 13 patients had a complete response after a long follow-up (120 months) while 6 patients relapsed, 2 in the allo and 4 in the autologous group.
The present analysis, although retrospective, suggest that allogeneic and autologous HSCT may induce a response in half of patient with severe refractory autoimmune cytopenia. Given the rarity of disease and the low number of transplant per center (1 over 10 years) it is high unlikely that a prospective study can ever be done. At the same time HSCT may be considered for patients with Evans syndrome, AIHA and ITP refractory to 2-3 lines of treatment under the "Clinical Option" criterion. If an HLA identical sibling is available, an allogeneic HSCT may be considered. Alternative donor from a well matched unrelated donor may also be considered in Evans syndrome. In the case that no compatible donor can be identified, autologous HSCT is an option.

P1188
Infectious complications during the mobilization phase in patients with refractory Crohn's disease suitable for autologous stem cell transplantation
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Background: Crohn´s disease (CD) is an inflammatory condition of unknown aetiology that can affect any portion of the gastrointestinal tract. Those patients who are refractory to conventional therapy or steroid-dependent are a challenging problem. As CD involves a loss of immune tolerance in the gastrointestinal tract, autologous stem cell transplantation (SCT) could be of value, similarly to other autoimmune diseases. With this aim, ASTIC (Autologous SCT International Crohn's Disease Trial) trial was designed. Mobilisation is achieved using cyclophosphamide 2 g/m² on 2 consecutive days followed by filgrastim 10 mg/kg/d 5 days later. We summarize the infectious complications observed during this procedure.

Patients and methods: Between 11/2007 and 11/2010, 15 patients with refractory CD (11 female/4 male) were mobilised. Median age was 30 y (range: 16-39). Nine of them fulfilled all inclusion criteria for ASTIC trial, and 6 did not: 3 because age <18 y, 1 for high body mass index and 2 because previous surgery invalidated assessment of CD activity index. Four had perianal disease and 4 an ileostomy with colon in situ. For the special characteristics of these patients and due to safety issues, the mobilisation was done in an in-patient basis. All they received low bacterial content diets and prophylaxis with levofloxacin and fluconazole. Additionally, piperacilin-tazobactam was started when neutrophils dropped below 1 x 10^9/L.
Results: In all cases an adequate number of CD34+ cells was collected (median: 9.8x106 CD34+ cells/kg (range: 5.3-20.1)). Median days of hospitalisation and neutropenia (<1 x 10^9/L) were 16 (range:12-33) and 6 days (range:5-7), respectively. Five patients remained afebrile during all mobilisation phase. The remaining 10 presented fever in relation to neutropenia, 7 of them with negative blood cultures and 3 bacteraemia due to Escherichia coli (1) and Klebsiella pneumoniae (2). Of these 3 patients, 2 presented septic shock and 1 reversible renal failure. The evolution was favourable in all them after adjusting antibiotic treatment.
Conclusions: Although this mobilisation scheme is highly effective, patients with CD have an extremely high-risk of severe infectious complications after mobilisation despite intensive preventive measures. A careful and intensive monitorisation should be applied in all cases to avoid additional causes of morbidity in these patients.

P1189
BEAM/horse-ATG is not different to CY/rabbit-ATG conditioning regimen regarding the development of CMV infection after autologous stem cell transplantation for multiple sclerosis
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Actually, tow conditioning regimens for autologous hematopoietic stem cell transplantation (AHSCT) have been used to treat Multiple Sclerosis (MS). CY/rATG seems to cause less toxicity than the BEAM/hATG without significant differences regarding to the effectiveness of each other (Hamerschlak N, et al, 2010). Besides that, there is no comparison of CMV infection between MS patients conditioned with those regimens. Thus, we decided to test the hypothesis that MS patients conditioned with BEAM/hATG are more likely to be associated with CMV infection than those conditioned with CY/rATG. Therefore, we studied a cohort of MS patients treated with AHSCT between August 2002 to December 2009 at our Institution. The incidence of CMV infection until D+60 after AHSCT was compared in 10 MS patients conditioned with BEAM/hATG against 50 MS patients conditioned with CY/rATG. All patients received the same mobilization regimen and were infused with more than 3 x 10^6 unmanipulated CD 34+ cells/kg. All blood components used were irradiated and leukocyte depleted . CMV infection was defined as a positivity of at least one pp65 antigenemia assay at any level. Patients with five or more positive CMV cells and patients in use of steroids with any number of positive CMV cells were treated with Gancyclovir. No patient developed CMV disease and or primo infection after AHSCT. The groups' characteristics were compared with Student's t-test or the Mann–Whitney test for quantitative variables and with the Chi-square or Fisher's exact test for categorical variables. Relative risk (RR) and confidence interval (CI) were calculated as the risk for development of CMV infection in relation to the exposure to the BEAM/hATG conditioning. The groups' characteristics are described in table 1. Among the BEAM/hATG group 5 out of 10 patients developed CMV infection whereas in the CY/rATG group 13 out of 50 patients developed CMV infection. The RR for CMV infection for patients conditioned with BEAM/hATG was 1,92 without significance [CI (0,88 to 4,18)]. We have supposed that BEAM/hATG could be more immunosuppressive than CY/rATG in MS patients treated with AHSCT regarding the development of CMV infection. However, at the cohort of MS patients studied there is no difference regarding the incidence of CMV infection between those conditioned with BEAM/hATG and those conditioned with CY/rATG.

Table 1: Characteristics of patients undergoing BEAM/horse ATG and CY/rabbit ATG conditioning regimens for the treatment of multiple sclerosis patients with autologous hematopoietic SCT in HCFMRP-USP

	BEAM/horse ATG	CY/rabbit ATG	p
n	10	50	
Age (years)—median	40 (30 to 52)	40 (18 to 59)	0.15
Male	6 (60%)	17 (34%)	0.12
Female	4 (40%)	33 (66%)	
Primary progressive	2 (20%)	4 (8%)	
Secondary progressive	6 (60%)	29 (58%)	0.08
Relapsing-remitting	0	17 (34%)	
Initial EDSS > or = 6.0	9 (90%)	30 (60%)	0.08
Initial EDSS <6.0	1 (10%)	20 (40%)	
CMV IgG before HSCT			
Positive	9 (90%)	35 (70%)	0.26
Negative	1 (10%)	15 (30%)	
CMV IgM before HSCT			
Positive	0	0	
Negative	0	0	

P1190
CMV and EBV reactivation in autologous haemopoietic stem cell transplantation for severe multiple sclerosis
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Introduction: Autologous HSCT is currently under investigation for the treatment of refractory, progressive MS. A better selection of patients and the use of less aggressive regimens resulted in a significant drop of Transplant-related Mortality (TRM). CMV and EBV tend to reactivate in ATG-treated allogeneic HSCT. We investigated the frequency of CMV/EBV viral load in a consecutive cohort of MS patients who underwent HSCT in our Center following BEAM and Anti-T Lymphocyte Globulin (ATG) conditioning regimen.
Patients and methods: 31 patients underwent auto-HSCT for severe MS from 1999 to 2010. All patients were mobilized by Cyclophosphamide 4 g/sqm + G-CSF and conditioned with BEAM plus rabbit ATG (Thymoglobulin©) at a dose ranging between 7.5 and 10 mg/Kg. Monitoring of CMV/EBV DNA by quantitative PCR on either circulating Mononuclear Cells (MNC) or Whole Blood (WB) was performed in 29 pts. In 18 patients ≥ 2 tests were available for both CMV and EBV from +20 and +120 days. Pre-emptive treatment was administered in case of viral DNA load >1x103 copies/105MNC or >1x104 copies/ml WB in two subsequent determinations.
Results: Overall 84 and 95 determinations were done for CMV and EBV, respectively. 10/29 patients (34.5%) were shown positive for CMV at a median of 33,5 days from HSCT (28-47), whereas 11/29 patients (37.9%) were positive for EBV at a median of 29 days from HSCT (20-109). Five patients resulted positive for both viruses. According to the ongoing protocols, 20% of CMV+ patients and 18% of EBV+ patients received a pre-emptive treatment with a fast drop of viral titer. In the other patients the reactivation was self-limiting in 2-3 weeks. One patient developed a severe CMV infection at + 47 days successfully treated the iv administration of Gancyclovir and Foscarnet. One EBV+ patient developed fever which responded promptly after Rituximab. Moreover, in 16/29 patients a sporadic determination of CMV/EBV was performed at early (between +5 and + 20) and late phases (beyond 120 days) without any evidence of viral reactivation.

Conclusions: Autologous HSCT with BEAM/ATG can be safely performed in MS patients. A significant reactivation of both CMV and EBV can occur in the 3 months following HSCT, possibly related to the ATG administration. Monitoring of viral load and, whenever necessary, pre-emptive anti-viral treatment should be performed, in order to improve safety of HSCT in this subset of non-neoplastic patients.

P1191

HSCT for severe multiple sclerosis with BEAM/ATG: impact on female fertility

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Introduction: Autologous stem cell transplantation (HSCT) has been increasingly used in the last 15 years for the treatment of severe Autoimmune Diseases (ADs). Transplant-related toxicity is a major issue in this type of non-malignant disorders, which do not usually result in a shortage of the life expectance. Multiple Sclerosis (MS) is the most frequent indication for HSCT in ADs, mostly in young women. Impairment of fertility is a common side effect of HSCT and therefore its assessment is particularly important in this specific subset of patients.

Patients and methods: We carried out a retrospective analysis of 24 women who underwent HSCT for severe MS in our centre from 1998 to 2010. All patients were mobilized by Cyclophosphamide 4 g/sqm and grafted with unmanipulated Peripheral Blood Stem Cells (PBSC) after being conditioned with BEAM and ATG. Women older than 45 years at HSCT were not considered, 2 patients have not answered to questionarie and 2 were lost at FU. Data were collected through a specific questionnaire aimed to determine the number of pregnancies, the incidence of amenorrhea before and after HSCT.

Results: Median age at HSCT was 34,5 years (range, 26–47) for males and 35,5 years (range, 21–44). Out of the 16 women included, 9 had a regular menstrual cycle before HSCT while 7 reported abnormalities due to previous treatments, such as Mitoxantrone. Eight women were younger than 35 years at HSCT, 7/8 reported a regular menstrual cycle prior to the transplantation. After HSCT 3 of them didn't recover they gonadal function at a median of 2 years median (range 6 months-4 years), whilst 2 reported a normalization with hormonal therapy at 6 and 9 months, respectively. Three patients (37,5%) spontaneously recovered their function. Six women were aged between 35 and 40 years at HSCT, 4/6 didn't' have any menstrual activity at baseline and didn't recover after HSCT, whilst two patients with normal cycles recovered properly at 2 and 6 months, respectively. No pregnancies were reported.

Discussion: Overall the incidence of amenorrhea is lower than in haematological patients, especially in young women treated early in the disease. This finding is expected to improve further as the current therapeutic approach in early diagnosed MS does not include cytostatic drugs, therefore decreasing the cumulative dosage. A wider survey in the EBMT database and a more focused endocrinological assessment is desirable in this peculiar subset of young patients.

P1192

Neuromyelitis optica remission after allo-SCT: results in 2 patients and correlation with T- and B-cell biomarkers of tolerance

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Background: Neuromyelitis optica (NO) or Devic syndrome is an autoinflammatory disease of the central nervous system within the multiple sclerosis (SM) spectrum; NO is characterized by the presence of aquaporin-4 antibodies AQP4, which possibly play a pathogenetic role. Current standard treatments often fail and prognosis of resistant cases is dismal.

Aim of this study was to investigate the use of allo-SCT to re-establish tolerance in NO.

Patients and transplant characteristics: Two cases of resistant NO (one 30 yrs old male, one 28 yrs female) were transplanted from a HLA-id sibling and a MUD, respectively. Previous treatment included high-dose corticosteroids, cyclophosphamide, rituximab, natalizumab, alemtuzumab, plasma exchange, high-dose Thiotepa/cyclophosphamide and/or BEAM/ATG/CSA with auto-SCT rescue. The conditioning regimen consisted of full-dose treosulfan, fludarabine, rituximab and ATG-Fresenius. GvHD prophylaxis was methotrexate and cyclosporine (HLA-id sib) or mycophenolate mofetil and rapamycin (MUD).

Results: Hematopoietic recovery occurred within day 30 with 100% donor chimerism in both patients. There was one episode of febrile neutropenia and one of CMV reactivation, both responsive to medical therapy. There was no GvHD. Both patients benefited from a marked improvement in neurological function. At six months, the Expanded Disability Score Status dropped from 6 (paraparesis) to 5 (deambulation) in the first patient, and from 8 (tetraplegia, bladder incontinence, visual impairment) to 7 (ability to move limbs), in the second. MRI did not show the appearance of new enhancing lesions. In the patient with longer follow-up (18 months), AQ4 autoantibodies became negative. Immune reconstitution was characterized by preponderance of naïve B cells (CD38+/CD27- range 86-91% vs controls 43-47%) and normalization of recent thymic emigrants (CD4+/CD45RA+/CD62L+/CD31+ 72-87% vs 61-96%) and Tregs frequencies (CD4+/CD25+/FoxP3+/IL-7Rα- 3.6-4.9% vs 1.7-5.2%).

Conclusions: Allo-SCT is a promising platform for the treatment of resistant NO and its efficacy associates with T- and B-cell biomarkers of a newly formed, tolerant immune system.

P1193

Development of antinuclear antibodies with new specificities during lupus relapse after autologous stem cell transplantation for severe systemic lupus erythematosus suggests de novo development of disease rather than lupus reactivation

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Introduction: In recent years, clinical trials have indicated that immunoablation followed by autologous hematopoietic stem cell transplantation (ASCT) has the potential to induce long-term clinical remission in patients with systemic lupus erythematosus (SLE). However, relapse of disease may occur in a fraction of these patients.

Methods: As part of a monocentric phase I/II clinical trial, seven patients have received immunoablation followed by CD34-ASCT for refractory SLE at the Charité – University Medicine Berlin. Peripheral blood lymphocytes were analyzed using multiparameter flow cytometry and serum titer and specificities of autoantibodies were monitored with ELISA.

Results: With a median follow-up of 84 months, clinical and serologic remission could be achieved in all patients. Three patients suffered a relapses of SLE after being free of clinical symptoms for 18, 30 and 80 months, respectively. From these three patients, one showed persistence of ANA in serum after ASCT, while two patients became negative for ANA after ASCT and redeveloped ANA prior to the flare. Interestingly, the ANA pattern in these patients changed during lupus flares compared to pre-ASCT: one patient developed antinuclear antibodies specific for Smith-Antigen (Sm) while anti-Ro/SSA and anti-La/SSB antibodies disappeared, the other patient newly developed anti-Ro/SSA and anti-La/SSB antibodies. In the patient with ANA persistence, antibodies specific for Ribosomal-P and RNP/Sm became negative after ASCT but returned 6 months prior to lupus flare.
Discussion: Our data show that immunoablation followed by ASCT has the potential to reinduce self-tolerance in SLE patients leading to long-term clinical and serologic remissions despite discontinuation of immunosuppressive treatment. However, self-tolerance is apparently not maintained in all patients. Based on serologic findings we propose that relapsed patients after ASCT for SLE can be dissected in those that show “reactivation of disease” maybe due to an insufficient depletion of the pathogenic immunologic memory and those that de novo develop SLE e.g. by the presentation of autoantigens in a tolerance-breaking form in genetically susceptible individuals, the latter being characterizid by the development of new pathogenic antinuclear antibodies. Further clinical studies are necessary to identify factors that predispose to the development of lupus flares after ASCT.

P1194

Risk assessment of high-dose immunosuppressive therapy with haematopoietic stem cell transplantation in patients with multiple sclerosis: a 10-year single-centre experience

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Purpose: To assess risk factors of HSCT in MS patients (pts).
Patients and methods: We have provide HSCT in 23 MS pts (11 male and 12 – female) since October 2000. There were 5 pts with primary progressive MS, 12 pts with secondary progressive MS and 6 pts with relapsing-remitting course of MS. Median age was 34,5 yrs (22-55 yrs). Median follow up was 4,5 yrs (1,5yrs-9 yrs 7 months). Median EDSS at the time of HSCT was 5,7 (1,5-7,5), at the last follow up 5,4 (1,0-8,0). The majority of patients had had previous 2 or 3 lines of immunosuppressive and immunomodulation therapy. Median time from disease onset till HSCT was 6,8 yrs.
We used myeloablative (BEAM) in 17 cases and non-myeloablative regimen (fludarabine+melphalane) in 6 cases. In all patients T-cell depletion in vivo by using of ATG 5 mg/kg on days +1,+2 was performed.
Results: Mean neutropenia duration was 12,7 d. Main complications in early posttransplant period were infections (19 pts), hemorrhage (12 pts), neurologic deterioration (7 pts) and serum sickness (10 pts). One patient died on day +8 from septicemia. In early posttransplant period (D+100) objective response (imp rovement+stabilisation) was 81%.
At last follow-up (June 2010) objective response was 62%. One pt died 2years after HSCT in an accident.
There were no statistically-valid differences between HSCT outcome and gender, age, course of MS, conditioning regimen. EDSS at HSCT (<6,5) and disease duration at HSCT (< 3,5 yrs) were statistically significant factors.
Conclusions: HSCT in MS is feasible procedure and significant majority of pts may at least stabilize their disability. The risk of the procedure can be minimized by detailed individual risk assessment before the inclusion.

P1195

Autologous haematopoietic stem cell transplantation in multiple sclerosis – the experience of the BMT Unit in Targu-Mures, Romania

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We present the results, complications and the observed benefits in 8 cases of multiple sclerosis patients who underwent autologous stem cell transplantation in the BMT Unit, Targu-Mures in the period 2009-2010.
All patients were in secondary progressive phase with EDSS score between 4-6. Mobilization was obtained with Cyclophosphamid + G-CSF due to the benefits of adding Cyclophosphamid because its immunosupressive effect, too. Conditioning treatment was the standard BEAM protocol. We had several infectious complications but none of them life-threathening and we had no mortality. In our patients inflamatory MRI activity was supressed visibly and the motor functions very dramatically improved and in one case urinary incontinence resolved also.
We consider autologous stem cell transplantation in multiple sclerosis beneficial due to the prolonged immunosupressive effect of the conditioning treatment and it can also have same beneficial effects on resetting the immune system.

P1196

Therapy-resistant autoimmune haemolytic anaemia following unrelated cord blood transplantation

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Background: Autoimmune hemolytic anemia (AIHA) is a rare complication of allogeneic hematopoietic stem cell transplantation allo-HSCT) with high mortality. For AIHA resistant to conventional treatment options, successful therapy with Rituximab, Sirolimus or Alemtuzumab has been reported. Here, we describe a clinical case of lethal AIHA after unrelated cord blood transplantation (UCBT) which did not respond to conventional therapy, Rituximab, Sirolimus and Alemtuzumab. Analysis of peripheral blood (PB) revealed a low frequency of naive T cells.
Patients and methods: A patient with α-mannosidosis who underwent UCBT from HLA-matched donor (6/6), with recipient/donor D-mismatch (donor: Rh-, recipient: Rh+). The control cohort (n=8) included patients with different hematological disorders, who underwent allo-HSCT and did not develop AIHA. The conditioning regimen was myeloablative for all patients. Anti-thymocyte globulin was given to recipients of unrelated stem cells. GVHD prophylaxis was Cyclopsorin A. AIHA was treated with steroids, high dose intravenous immunoglobulin, Rituximab, plasmapheresis, Vincristine, Cyclophosphamide, splenectomy, Tacrolimus, Sirolimus and Alemtuzumab. PB samples were analysed by polychromatic flow cytometry. Regulatory T cells (Treg) were identified as CD25high CD127-/low events. CD45RAhighCD62L+events were considered to be naïve T cells.
Results: Three months after transplantation, the patient developed AIHA. Antibodies were initially directed to the D antigen. Thereafter, red blood cell antibodies with pan-agglutinating properties appeared. Conventional therapy protocols failed to revert autoimmunity. In addition, Rituximab, Sirolimus and Alemtuzumab were unable to stop autoimmunity. Eight months after transplantation, necrotizing vasculitis developed and the patient died one month later. By immunophenotyping, naïve T cells were found at very low frequency 6 months after transplantation. In control samples, the frequency of naïve T cells and naïve Treg was highly variable and no statistically

significant difference was found compared to the patient with AIHA. Likewise, the frequency of Treg was similar in the patient with AIHA and control samples.
Conclusion: This patient developed AIHA following allo-HSCT which was resistant to all treatment efforts. The low frequency of newly produced naïve Treg may have favored the lack of control of autoimmunity, although no significant difference compared to the controls was observed.

Solid tumours

P1197

Temporary remission of lung metastases of an osteosarcoma demonstrated by HLA antibody staining of infiltrating T-lymphocytes of the mother following KIR-lig- and mismatched haplo-identical stem cell transplantation

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Introduction: Despite progress in conventional treatment and surgical procedures osteosarcoma remains still one of the most challenging tumour entities. Patients with lung metastases at diagnosis and poor response to chemotherapy have the worst prognosis. In these patients alternative strategies such as stem cell therapies to induce an allogeneic graft versus tumour effect have been attempted.
Patient and methods: A 10 year old female with osteosarcoma of the right distal femur with multiple metastases in both lungs, was treated according to the EURAMOS 1/COSS Study protocol. After 6 cycles of neoadjuvant chemotherapy the complete femur was replaced by an endoprothesis. Histopathological evaluation revealed regression grade III and tumour free resection margins. After completion of adjuvant chemotherapy resection of more than 20 pulmonary metastases was attempted. The majority of these metastases showed viable tumour tissue. Despite introduction of antiangiogenetic treatment with pegylated Interferon the patient showed progression of the lung lesions. The patient was then conditioned with Fludarabine/Thiotepa/Melphalan/OKT3 and transplanted with 15.4 x 10^6 CD3/19-depleted stem cells of her KIR-ligand mismatched haploidentical mother. Beginning on day +75 the patient received 33 donor lymphocyte transfusions starting with 1 x 10^5 up to a maximum number of 1 x 10^6 lymphocytes/kg body weight. On day +194 seven remaining lung metastases were resected. In 6/7 tumour samples no viable tumour cells could be detected. All these specimens showed a distinct infiltration and a peripheral ridge of haploidentical CD4/8+ T-cells demonstrated by HLA antibodies against the HLA-type of the mother. Thus the haploidentical immune system boosted by repeated donor lymphocyte infusions was able to maintain a very good partial remission for almost 1 year. At day +355 CT-scan revealed reappearance of metastases in both lungs and a bone scan showed local tumour recurrence at the primary site.
Conclusion: The introduction of the donor`s immune system seemed to be able to control the tumour in this patient up to one year. Recurrence of lung metastases might also have been caused by local relapse of osteosarcoma. The activation of allogeneic adaptive T-cells appeared to be at least capable to maintain a temporary remission on the before refractory lung metastases.

P1198

Retrospective analysis of treatment-related toxicities and outcome in high-risk Ewing sarcoma patients receiving PO or IV busulfan-based high-dose chemotherapy with autologous stem cell transplantation

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Objectives: Busulfan (BU), in combination with melphalan (MEL), high-dose chemotherapy (HDC) with autologous stem cell transplantation is widely used in consolidation treatment of poor-prognosis Ewing sarcoma patients. Intravenous (IV) BU was approved for application during the EURO-E.W.I.N.G.99 (EE99) trial. We analyzed treatment-related toxicities and outcome of BU administered orally (PO) or IV in patients treated from 1999-2009 according to the EE99 protocol.
Methods: Administration method and doses of busulfan given to 157 patients (pts) treated with BU-based HDC (BU-MEL) were extracted from patient records and the EE99 trial database of the German Society of Pediatric Hematology and Oncology (GPOH). 113 pts had received PO BU; 32 pts had high-risk localized disease mainly for poor response to initial chemotherapy (R2loc; 28.3%), 31 pts pulmonary metastases (R2pulm; 27.4%), and 50 pts had primary mainly skeletal dissemination (R3; 44.2%). 44 pts had received IV BU (R2loc: 18; 40.9%; R2pulm: 9; 20.5%; R3: 17; 38.6%). HDC toxicity was analyzed by descriptive statistics according to modified CTC toxicity grade scales of the EE99 protocol. Outcome was analyzed descriptively by event-free-survival (EFS) and overall-survival (OS) controlled for risk factors by multivariate regression analysis.
Results: Grade 3 & 4 toxicity ratings occurred in 480 of 1759 cases (27.3%) in the PO group, and in 174 of 741 cases (23.5%; p=.048) in the IV group. Most of these serious toxicities were hematological with no difference in both groups (431 cases; >85% of patients per group; p=.59). Major differences with a clinical relevant reduction of more than 10% in single scales were observed in the IV group regarding: general condition (IV: 18.2% vs PO: 33.3%; p=.028; non-dichotomous), stomatitis (51.2% vs 63.2%; p=.032; non-dichotomous); diarrhoea (0% vs 12.5%; p=.033) and elevated bilirubin (2.8% vs 13.3%; p=.104). 5y-EFS (OS) was 0.47 (0.57; SE=.08) for IV BU vs 0.42 (0.49; SE=.05) in PO BU. Adjusted for risk group (R2loc; R2pulm; R3) and age (cut-off 15years), the EFS risk ratio for PO vs IV BU was 1.35 (95%CI 0.82-2.22), and the OS risk ratio was 1.49 (95%CI 0.85-2.61).
Conclusion: Results from this retrospective analysis of a large cohort of high-risk Ewing sarcoma patients indicate a reduction in toxicity with IV BU-based HDC.
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P1199

Autologous tumour-specific cytotoxic T-lymphocytes in osteosarcoma patients: a preclinical model

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Osteosarcoma (OS) is the most frequent bone sarcomas in young adults. Unresectable or metastatic presentations are currently characterized by an extremely severe prognosis, with a consequent great need for new therapeutic approaches. We conducted a preclinical study to investigate the potential efficacy of autologous tumor-specific cytotoxic T lymphocytes (CTL) as adoptive immunotherapy for bone sarcomas.

CTLs are a homogenous subset of ex-vivo expanded T lymphocytes and are endowed with a MHC-restricted antitumor activity.
We successfully generated autologous tumor cells and autologous tumor specific-CTLs from 6 patients with metastatic bone sarcomas.
CD8+ cells isolated from PBMCs were stimulated with autologous DCs loaded with autologous tumor cells in RPMI with 5% human serum (HS) supplemented with recombinant human interleukin-7 (rhIL-7), recombinant human interleukin-12 (rhIL-12) and recombinant human interleukin-15 (rhIL-15) for 7 days.
CTL obtained were expanded in an antigen independent manner by co-culture with irradiated allogenic PBMCs in RPMI with 5% HS supplemented with rhIL-2 and Muromonab-CD3 (OKT3).
The median ex-vivo expansion of CTLs, was 12 fold (10-18).
Cytotoxicity experiments demonstrated the potent and specific killing activity of CTLs against the autologous tumor cells as showed in figure 1. The tumor-specific cytotoxicity of CTLs was confirmed by the absence of any significant killing against PBMCs allogeneic and other OS tumor cell lines (ATCC).
Our data are the first report of antitumor activity of CTLs against autologous OS cells.
The interferon-γ release, valuated with test ELISPOT, corfimed the CTL specificity (figure 2).
Our findings are encouraging and support the designing of adoptive immunotherapy clinical trials with autologous CTLs for OS patients.

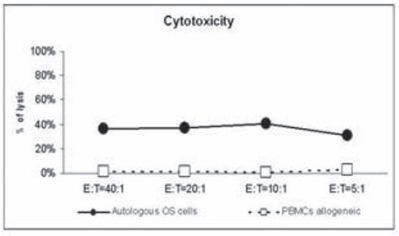

Figure 1

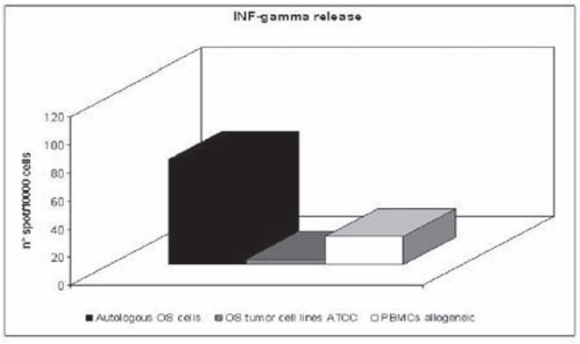

Figure 2

P1200
Employing in vivo bioluminescence imaging for assessing the contribution of TNF-TNFR interactions to tumour metastasis in a syngenic mouse model
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The cytokine tumor necrosis factor-α (TNF) has pleiotropic functions both in normal physiology and disease. TNF signals by activating two cell surface receptors TNFR1 and TNFR2. TNFR1 is expressed on most cells whereas TNFR2 is mainly expressed in cells of the hematopoietic system. TNF-TNFR interactions were shown to play a major role in graft-versus-leukemia effect and in the immunosurveillance of solid tumors. On the other hand, experimental data indicates that TNF might also promote tumor metastasis either directly by its pro-inflammatory actions or by promoting extravasation of tumor cells. We are interested in whether manipulating the TNF-TNFR-system of stem cell grafts could be beneficial in the treatment of malignant diseases.
We first set out to assess the direct influence of the TNF-TNFR-system on tumor metastasis. Here, we made use of the syngenic B16 melanoma mouse model combined with in vivo bioluminescence imaging. Firefly luciferase-transgenic B16 meanoma cells were injected intraveneously into syngenic albino C57BL/6 hosts. The host mice were either of wildtype, TNF knockout or TNFR1 knockout genotype. The localization and expansion of the B16 cells was monitored every other day by in vivo bioluminescence imaging for up to 22 days. On days 15 and 22, mice were sacrificed and a wide variety of organs as well as additionally observed tumors were imaged ex vivo to further elucidate the organ-specific tumor burden.
B16 tumors were primarily found in the lungs of all genotypes. We found both TNF knockout and TNFR1 knockout mice to be more susceptible to B16 melanoma cells in terms of lung metastases than wildtype mice. In the two knockout mice a luciferase signal could be observed earlier, the signal intensity was more pronounced over the course of the experiment and also the signal intensity in the lungs as assessed with ex vivo imaging was more pronounced.
This shows that TNF-TNFR interactions are an important step in tumor metastasis. Targeting this signalling system in stem cell grafts might be helpful in terms of treating metastatic tumors.

P1201
Short-term dendritic cell generation for metastatic solid tumours
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Extracorporeal photochemotherapy (ECP),initially born to treat cutaneous T cell lymphoma has become an accepted therapeutic strategy for graft versus host disease and for solid organ rejection. Edelson successively developed the concept that collected monocytes by leukapheresis after UVA irradiation and overnight incubation can mature into dendritic cells(DCs). This challenging concept can open new possibilities in the field of immunotherapy. We developed a protocol of vaccine therapy employing ECP technique to collect large quantity of mononuclear cells from metastatic end stage patients and incubate overnight the collected cells after UVA irradiation in presence of autologous tumor lysate (Transimmunization-TI).The aim of the study was to establish the safety and feasibility of TI and to demonstrate the transformation of the collected monocytes into DCs loaded with tumor antigens.ECP procedures were performed with the “French method” employing the Spectra Cobe cell separator.Irradiation in presence of 8-MOP was carried out by Vilbert Lourmat-Macogenic device. Finally the collected cells were transferred into a gas permeable bag fit to platelet storage, incubated at room temperature in presence of autologous tumor lysate and reinfused to the patient after 24 hours.Microbial tests were performed at time of collection and reinfusion..All 10 pts (5 males and 5 females) enrolled, after informed consent and approval of ethical committee, suffered of various metastatic tumors except 1 affected with resistant T cell lymphoma.No relevant side effects were registered also in very compromised patients. Microbial tests were always negative. After a mean follow up of 14 months, 5 patients died for disease progression after a mean treatment time of 5 months, 4 pts are alive with a slow disease progression and 1 patient (T cell cutaneous lymphoma) is alive and well..After overnight incubation, a very high cell viability and MNC purity (mean 82 and 92% respectively) were documented together with a high monocyte

content (on average 2.3x10e9).Moreover, we observed the possibility to generate a higher % of DCs from the monocytes of patient treated with T.I. compared with normal donors and previously chemo-treated pts In conclusion TI appears to be a safe and feasible procedure with the potential to be a short-term "ex-vivo"large scale DCs generation system that may prime an efficient network of immune stimulation in tumor patients.

P1202

Haematopoietic stem cell mobilization with plerixafor in patients with non-haematological diseases

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Autologous haematopoietic stem cell transplantation (ASCT) can be a curative procedure for a variety of malignancies. Successful ASCT is dependent on transplantation of sufficient CD34+ cells to ensure prompt and durable engraftment. However, a number of patients fail to mobilize the traditional threshold of >2x10E6/kg CD34+ cells. Recently, the CXCR4 antagonist plerixafor was shown to be safe and effective for haematopoietic stem cell (HSC) mobilization from patients with myeloma and lymphoma. This report aimed to evaluate the effect of plerixafor on HSC mobilization in patients with non-haematological malignancies in whom data regarding pretreatment, mobilization and CD34+ cell yield were available. Thirty-two patients who previously failed HSC mobilization were included in this study. Patients were diagnosed with germ cell (n=11), Ewing sarcoma (n=5), Wiscott Aldrich disease (n=5), neuroblastoma (n=3), medulloblastoma (n=2), and others (n=6). Median age was 26 (range, 2-70) years, and 78% were males. The median number of prior chemotherapy regimens was 2 (range, 0-5), with 22% of patients receiving prior radiotherapy. In the majority of patients the mobilization regimen consisted of G-CSF + plerixafor, whereas a few patients received chemotherapy followed by G-CSF + plerixafor. On the first apheresis day (about 10-11 hrs. after Plerixafor), the peripheral blood CD34+ cells count reached a median of 34 (range, 2-250) CD34+ cells/µl. Twenty-five of 32 (78%) patients achieved the target cell dose of 2x10E6 CD34+ cells/kg (median 5.2, range 2-29.53 x10E6/kg CD34+ cells), while 7 patients failed to collect a sufficient cell dose (median 1.5, range 0.33-1.8 x10E6/kg CD34+ cells). At last follow-up, 16 patients (50%) underwent ASCT and received a median of 3.6 (range, 2.6-6.7) x10E6/kg CD34+ cells.
In all, these data suggest that plerixafor + G-CSF ± chemotherapy is effective in patients with non-haematological diseases. In patients eligible for ASCT who have failed prior mobilization attempts, plerixafor can provide an opportunity to still pursue a potentially curative procedure.

P1203

Incorporation of taxanes in salvage and high-dose chemotherapy and autologous peripheral blood stem cell transplantation in patients with advanced germ cell tumours

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Paclitaxel-based combinations have been tested for standard salvage chemotherapy and in combination with HDCT. We include docetaxel in salvage regimens prior to high dose chemotherapy.
38 procedures of HDCT and ASCT were performed in twenty five relapsed or refractory germ cell cancer patients. 48% of patients were heavily pretreated with ≥2 lines of previous systemic therapy and median of 7,4 cycles of cisplatin-based chemotherapy. 16 patients were cisplatin refractory and 9 patients had relapse. Treatment plan consisted of 2 cycles of salvage chemotherapy and two sequential cycles of high dose etoposide (1500 mg/m2) and carboplatin 1800 mg/m² (24 AUC) with stem cells support.
Salvage regimens were docetaxel (160 mg), ifosfamide (6 g/m²) and cisplatin (100 mg/m²) in 13 patients, standard TIP in 4 patients, GOP (gemcitabine, oxaliplatin and paclitaxel) in 4 and VIP in 4 patients, depending on previous therapy or drugs availability. Response was achieved in 21 (84%) patients, partial in 17, complete – in 4 patients, 4 had stable disease. Stem cells were successfully collected in all patients, but 5 of them required second mobilisation. Eleven patients received two high dose regimens, 13 patients – one, and 1 patient received 3 cycles of HDCT. High-dose paclitaxel was included in high dose regimen in 5 cases.
Results: The median age was 27,5 years (range 18 to 41). The most frequent non-hematological toxicity was: nausea and vomiting, mucositis and hepatic toxicity. Transplant related mortality was 4 %, one patient died due to renal insufficiency after HDCT. At a median follow-up time of 4,9 years (2 – 111,5 months), a continuously disease-free status was achieved in 12 patients, in 5 patients residual tumor were removed surgically. Nine patients died because of disease progression. Two are live in relapse. Disease-free and overall survivals at 5 years are 51 and 60%, respectively.
Conclusion: incorporation of docetaxel and paclitaxel in salvage treatment and HDCT appears to benefit patients with resistant germ cell tumors with manageable toxicity.

P1204

High-dose chemotherapy with autologous rescue for treatment of retinoblastoma. A preliminary single-centre experience

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Introduction: Retinoblastoma (RB) is the most common intraocular malignancy developing in cells that have cancer-predisposing mutations in both copies of the gene RB1. It occurs in children, usually before age five years. About 40% of them have bilateral RB, diagnosed at a mean age of 15 months, and also have an increased risk of developing other RB-related (non-ocular) tumors.
Treatment options depend on tumor stage, number of tumor foci (unifocal, unilateral multifocal, or bilateral), localization and size of the tumor(s) within the eye, presence of vitreous seeding, and age. They may include enucleation, cryotherapy, photocoagulation, photochemistry, radiation therapy by external beam or episcleral plaques. In the attempt to spare the eye, newer options have been developed which include systemic chemotherapy combined with, or followed by, local therapy. If possible, high-dose radiotherapy should be avoided to reduce orbital asymmetry and lifetime risk of developing late-onset secondary cancers.
The use of high-dose chemotherapy with autologous stem cell rescue (HDCT/ASCR) is a long-lasting stand-point the treatment of high-risk solid tumor, in which this therapeutic approach may provide an improved outcome. As RB is a chemosensitive tumor, a strategy using HDCT/ASCR might be effective in the treatment of bilateral advanced RB (Lee, Bone Marrow Transplantation, 2008).
Patients and methods: starting from 2008, all children with bilateral RB were treated with chemotherapy and local therapy followed by consolidation with Tandem HDCT/ASCR strategy. Conditioning regimens: CTE (Carboplatin 500 mg/m²/day, days -8 -7 -6; Thiotepa 300 mg/m²/day, days –5- 4-3; Etoposide 250 mg/m²/day, days –5,-4) at the first course; CY 1500 mg/m²/day, days –8-7-6-5, and Melphalan 60 mg/m²/day, days -4, -3, -2) at the second course. We treated 4 children, age 10 to 30 months,

with bilateral RB (n=3) or unilateral RB with CSF involvement. All children engrafted for PMN (median, day +12) and PLT (median, day +13), with mild toxicity (mucositis II-III, diarrhea and skin toxicity).
All 4 had a major response: three completed the program and achieved no evidence of disease, the fourth is during second transplant.
Conclusion: in our preliminary experience, HDCT/ASCR had a limited toxicity and was effective in obtaining disease control in patients with bilateral or high-risk RB. This regimen allowed to spare enucleation, and the risk of late morbidities associated with radiotherapy.

P1205

High-dose chemotherapy and autologous stem cell transplantation in paediatric Ewing's sarcoma/PNET

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5-year event-free survival (EFS) for patients with localized pediatric Ewing's sarcoma/PNET is 70-80%, but in high-risk group (metastatic disease, unfavorable localization or large initial tumor mass) it is still 20-30%. High-dose chemotherapy (HDCT) with autologous hematopoietic stem-cell transplantation (auto-HSCT) is considered a promising option in patients with metastatic disease. This study was aimed at assesing possible survival benefit for this patient population.
Patients and methods: From 2003 to January 2010 23 pediatric patients received therapy for high-risk Ewing's sarcoma/PNET. Male-female ratio was 2,9:1, median of age at diagnosis 12,2 years. 18 of the patients had multiple metastases, 12 patients initially had a large tumor volume and unfavorable tumor localization. All patients received 6 courses of inductive polychemotherapy (ifosfamide, vin-cristine, doxorubicin and etoposide), surgical treatment or local irradiation (48 -56 Gy). Furthermore, 8 patients 8 courses of maintenance VAC chemotherapy and 15 patients received HDCT with auto-HSCT. At the times of transplant 8 patients were in complete remission (CR) and 7 patients were in partial remission (PR). Conditioning regimen consisted of busulfan 16 mg/kg and melphalan 140 mg/m2 (n=16). Stem-cell sources used were bone marrow (n=8), peripheral blood stem cells (n=3) or both (n=5). Mean CD34+ cells dose was 3,05 x 106/kg.
Results: In the maintenance therapy group (n=8) 7 patients had a relapse within 2–20 months after the end of treatment (5 patients with metastatic disease on re-lapse died, 2 patients with local relapse received salvage therapy and are now alive), 1 patient died of sepsis. In the HDCT group (n=15) 13 of the patients are alive (12–49 months after HDCT), 7 of them remain in remission. All except 2 children with PR at times of transplant had early relapses (32–128 days after HSCT). 5 of 8 patients with CR at time of transplant are still in remission, 3 had late relapses (365-628 days after HSCT).
Conclusions: HDCT with auto-SCT significantly lowers relapse rate in pediatric patients with high-risk Ewing's sarcoma/PNET, which achieved CR after induction chemotherapy. The growing rate of late relapses suggests the necessity of post-HDCT therapy.

P1206

Lower incidence of treatment-related mortality in reduced versus high-intensity conditioning for allogeneic stem cell transplants in paediatric solid tumour

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Objectives: In Solid Tumors (ST) patients treated with allogeneic stem cell transplantation (allo-SCT), reduced intensity (RIC) and high intensity conditioning (HIC) regimens heve beenanalyzed regarding toxicity, relapse, survival (OS) and Graft-versus-Tumor effect (GvT).
Methods: We performed a retrospective analysis of 24 ST patients consistent in 12 neuroblastoma (NB), 8 Ewing's sarcoma (ES) and 4 rhabdomyosarcoma (RMS) treated in our Institution with allo-SCT between 1995 and 2010. 20 patients were male and 4 female. 14 patients received RIC (group A) and 10 patients received HIC (group B). Median age at allo-SCT was 11 years (range 3-21 years). 18/24 have relapsed after a previous auto-SCT. 4 patients have been transplanted in complete remission (CR) and 12 in partial remission (PR) and 8 in progressive disease (PD). The donor was related in 10/10 patients of group B and in 8/14 in group A. 6/14 donors of group A were unrelated. SC source was bone marrow in 3/10 patients in group B and 9/14 in group A. Age and stage distribution did not differ significantly between both groups.
Results: Median overall follow-up (FU) was 12 months (range 2-179 months) for group B and 7 months (range 2-31 months) for group A patients. After a median FU of 12 months in the group B patients the treatment related mortality (TRM) probability was 20.5%(16.7). 2/10 patients died for TRM, 7/10 relapsed and died while 1 is alive and well. After a median FUP of 7 months the TRM probability in the group A patients was 0, 6/14 patients relapsed and died while 8 were alive and well. No association between HLA mismatch and GvT has been observed in this analysis.
Conclusion: In this retrospective analysis, there was a lower incidence of TRM in RIC versus HIC for allo-SCT in ST patients and an apparently better outcome. A longer FU in group A patients needed to evidence a clinically relevant GvT with RIC protocols.

P1207

Autologous stem cell transplantation for high-risk neuroblastoma: the Iranian experience

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Introduction: Neuroblastoma (NBL) is the most common extracranial, solid tumor in children, accounting for 8% to 10% of all childhood cancers. Significantly, autologous transplantation appears to have the largest impact on the survival of the high risk subset of patients.
Patients and methods: This study includes high risk, relapse, or refractory patients with NBL who underwent Stem Cell Transplantation (SCT) from 1998 to 2010. There were 15 patients with NBL consisting of 8 males and 7 females. The main conditioning regimen consisted of Carboplatin (400 mg/m2 for 3 days), Etoposide (200 mg /m2 for 3 days), and Melphalan (75 mg /m2 for 2 days). Since 2008, MIBG test has been performed in all the patients, one month before transplantation. Then, those patients who had positive MIBG test received MIBG 12 (mci/kg) with the same conditioning regimen. From that time (2008) all of the patients began receiving 13-cis-retinoic-Acid 120-160 mg/m2/2weeks per month, as maintenance from day sixty after SCT until one year later.
Results: The median age of recipients was 5 years (range: 2-10 years). The source of stem cells was peripheral blood in 13 patients and bone marrow in 2 patients. The median time to

an Absolute Neutrophil Count ≥0.5*10^9/L was 11 days (range: 8-14 days). The median time to an Absolute platelet count of ≥20*10^9/L was 19 days (range 10-32 days). The median follow-up time was 5 months (1.5 month-5 years). 9 of the 15recipients had relapses. At the present time, 7 patients are still alive. One of them has relapse and the remaining 6 patients are in complete remission. Relapse was the only cause of death. It should be noted that 5 patients received MIBG 12 (mCi/kg) with the same conditioning regimen.
Conclusion: Considering the results of this study, we can conclude that SCT is a feasible and effective method of treatment for patients with high risk neuroblastoma exhibiting resistance to chemotherapy. Furthermore, adding MIBG therapy to the conditioning regimen might enhance the effect of treatment.

P1208
Madrid, 2nd International Workshop for Haplo-identical Stem Cell Transplantation in Paediatric Solid Tumours
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The Second International Workshop in Haploidentical stem cell transplantation in paediatric solid tumours was celebrated on July 2010 in Madrid, Spain. First meeting was celebrated on July 2009 in Brescia, Italy. This time the aim was discuss about the feasibility to develop a European pilot study for “Haploidentical stem cell transplantation for childhood refractory and metastatic solid tumours”.
Because prognosis for relapsed or refractory paediatric solid tumours is still poor, it guarantees innovative approaches. Intensive treatment regimen using higher doses of chemotherapy and autologous stem cell rescue has been increased toxicity and risk of second malignancy without improving event free survival and overall survival. However mainly experience acquired from adults cancer with conventional allograft has been disappointing.
The expanding knowledge of tumour and innate immune system crosstalk may provide clues for new antitumor cell therapy strategies. Innate immune system immunotolerance mediated by Natural Killer (NK) cell has been described in metastatic and refractory solid patients. Moreover majority of paediatric solid tumours, particularly if advanced stage, exhibit complete/partial absence of NK cell killer immunoglobulin like-receptor ligands and increase ligands through activatory NK receptors as NKG2D or DNAM-1. Furthermore, exquisitely sensitive to expanded NK cell lyses has been described in most of paediatric solid tumours. Because haploidentical stem cell transplantation (HP) constitute the best allograft platform to explode NK cell alloreactivity, we hypothesize it could be a novel tool to eliminate non-curable malignancies with conventional treatment. We also know single European paediatric centre has clinical trials ongoing with HP and alloreactive NK cell boost infusion. We suggest we should create a European consortia multicentre clinical trials aiming at the validation of NK alloreactive haploidentical stem cell transplantation as a novel therapeutic strategies for incurable paediatric solid tumours, by transnational research, and apply for Heath Framework Cooperation Programme for Research and Technological Development. Third edition will take place in Leiden, Netherlands, with lots of scopes and challenges.

P1209
High-dose chemotherapy with autologous stem cell support for the heavily pretreated patients with germ cell tumours: a Serbian single-centre first-experience report
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Purpose: The prognosis of heavily pretreated patients with germ cell tumors is poor. Long time survival can be however provided using high dose chemotherapy for small number of patients. We present our first results in high dose therapy for patients with germ cell tumors.
Patients and methods: From august 2008 to October 2010, we performed 9 transplantations in 6 highly refractory patients. All patients were male, median age was 34 years (22-48). Three patients had double transplant, two had only one because of progression, and for one patient second transplant is ongoing. Five of six patients had non-seminoma germ cell tumor at initial diagnosis time (3 teratoma, 2 embryonal carcinoma) and one patient had seminoma. Median time from diagnosis to first graft was seven years (3-18). Median number of prior therapy lines before mobilization protocol were 4 (3-4).
We performed, according to the Tenon Hospital Program (Tenon Hospital, Paris, France), stem cell mobilization using a combination of paclitaxel/epirubicin plus G-CSF. High dose therapy protocol was Carboplatin AUC 4 plus Etoposide 300mg/m² day 1-5. Patients recieved median number of 3.1x10^6 (range 1.9-4.5) CD34+ cells/kg of BW per transplant.
Results: There was no treatment-related mortality. The most common, grade 3/4 treatment related toxicities were: neutropenic fever (100% of patients), thrombocytopenia (100%), colitis/diarrhea (66%), nausea (55%), and oral mucositis (44%). There was one veno-occlusive liver disease, and two grade 4 infections (one facial phlegmona and one sepsis).We didn’t observe any CRs, we had 2 PRs, 2 SDs and 2 PDs. Median progression free survival time was 9.5 months (range 2-18). Three patients are still alive.
Conclusion: High dose chemotherapy in heavily pretreated patients with germ cell tumors is feasible, with a moderate progression free survival. Earlier treatment intensification in those patients is mandatory.

Experimental stem cell transplantation and Tumour stem cells

P1210
Allogeneic transplantation in elderly patients with poor prognosis acute myeloid leukaemia or myelodysplastic syndrome; the San Raffaele Institute experience.
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Background: allogeneic (allo) stem cell transplantation (SCT) is the only curative strategy for poor prognosis acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS). Patients (pts) older than 60 are not routinely offered an alloSCT as the procedure is considered highly toxic. Since 2002, 45 pts older than 60, with high risk AML/MDS, received an alloSCT from a matched related (MRD), matched unrelated (MUD), mismatched related (MMRD) or cord blood (CB) donor, at our Institute. Data on feasibility and outcome are here reported.
Aim: retrospective evaluation of feasibility and efficacy of alloSCT in elderly AML and MDS pts, with poor prognosis disease.

Methods: period 10/2002 to 11/2010, 45 pts, median age 64 (60-72). Diagnosis: de novoAML 16, sAML 13, therapy-related AML/MDS 4, MDS 12. Donors: MRD 10, MUD 9, MMRD 24, CB 2. Disease status at SCT: CR1 12, CR2 9, upfront 7, refractory 8, relapsed 9. Conditioning regimens contained fludarabine in 44 cases, treosulfan in 40, melphalan in 3, thiotepa in 2, cyclophosphamide and busulfan in 1 case, ATG in 34 cases. GvHD prophylaxis: T-cell depletion in 11 cases, CSA/MTX in 24, rapamycin/MMF in 10. All pts received SCT from peripheral blood.
Results: of 40 pts evaluable at day +30 38 (95%) were in CR, included 21 out of 26 (81%) not in CR at SCT. TRM within day +100 was 20% (9 pts), overall 44% (20 pts), for pts in CR was 43% (9 pts), for pts not in CR 46% (11 pts). Causes of TRM: 13 infections, 6 GvHD, 1 myocardial infarction. GvHD: acute 22% (9/40 pts), chronic 35% (8/23 pts). Relapses: 12 (31.5%). At last follow-up (FU) 16 pts (36%) are alive in CR, with a median FU of 617 (30-1659) days. Median EFS from SCT of all pts is 227 (3-2289) days. Of 14 pts older than 65, 4 (28.5%) are alive in CR, median EFS is 159 (14-2289) days.
Conclusions: alloSCT is feasible in elderly pts with poor prognosis AML/MDS, and expected OS <15%. TRM is equally distributed before and after day 100. This suggests that early toxicities due to the conditioning regimens are limited, and prolonged immunesuppression is responsible for a consistent proportion of fatal complications. Long term survival has been obtained, also for pts transplanted with active disease. In conclusion, new reduced-toxicity conditioning regimens, mainly fludarabine/treosulfan based, permit to offer an alloSCT to elderly pts with a dismal prognosis, also from alternative donors. Trials are ongoing to improve the management of GvHD and infections.

P1211
Fractioning of bone marrow mononuclear cells for treatment of myocardial ischaemic injury (experimental model)
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In view of the myocardium's limited capacity to regenerate spontaneously after an ischemic injury, the therapeutic use of exogenous progenitor cells has recently gained increasing interest. Our group developed a method of obtaining bone-marrow progenitor cells using differences in the rate of cell adhesion. Four weeks after the transepicardial injection of autologous non-adhering progenitor cells (NAPC) we observed the morphological evidence of significant increase in process of angiogenesis in a dog chronic ischemic heart disease (CIHD) model.
All experiments were approved by the local institutional ethics committee. 9 dogs, both sex, 1.5-2 years old, weighting 15-20 kg, were anesthetized intravenously, and artificially ventilated. A CIHD model was formed by ligation left coronary artery and its branches. Formation of MI was confirmed by ECG. Three months later bone marrow specimens were extracted from iliac bones. Bone marrow mononuclear cells (BMC) washed twice, resuspended in depleted nutrient solution and incubated in culture flasks. 10^7 NAPC (6 dogs) and BMC (3 dogs) were used for application around of MI zone in each case. Approximately 4 weeks after cells implantation, hearts were removed and fixed in 4% formalin for histochemical analysis. Myocardium of the left ventricle and apex of the heart was taken for microscopic examination. RT-PCR analysis was used for estimation of VEGF-A, -B, -C, -D and osteopontin mRNA expression level in NAPC and BMC.
The most significant manifestation of morphogenesis induced by implantation of BMC was the widespread ossification of epicardial and sub-epicardial layers of myocardium with randomly oriented bone beams. Van Gieson staining showed the presence of small bony beams that could be enveloped by multicore osteoclast cells and were subjected to partial resorption. Implantation of NAPC doesn`t lead to such side effects. To determine differences in the formation of the microvascular bed, approximately 800-1200 vessel profiles were analyzed per animal. The count of vessels with a diameter up to 40 micrometers stained isolectin GS-IB4 showed significant differences in NAPC compared with BMC implantation areas (2970 and 2120 vessels/mm² respectively). The levels of VEGF-D,-B and -A were significantly higher in NAPC group, but the level of VEGF-C and osteopontin was higher in BMC group.
So we demonstrated advantages of non-adherent mononuclear cells use in the treatment of ischemic myocardial injury.

P1212
Allogeneic haematopoietic stem cell transplantation in a patient with mitochondrial neurogastrointestinal encephalomyopathy: results of a six-month follow-up
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Objective: To describe the preliminary results of treatment with allogeneic hematopoietic stem cell transplantation (HSCT) in a patient affected by MNGIE.
Background: Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE) is a life-threatening autosomal recessive disorder, caused by a mutation in the gene of thymidine phosphorylase (TP) on chromosome 22q13.32-qter that leads to deletions and/or depletion of mitochondrial DNA. MNGIE is clinically characterized by gastrointestinal dysmotility, cachexia, ptosis, external ophthalmoplegia, polyneuropathy, and leukoencephalopathy. MNGIE is slowly progressive, potentially lethal and patients usually die in early adulthood. The therapeutic options are limited. We describe the case of a patient with MNGIE treated with HSCT.
Case report and results: A 21 years old female patient was referred to electromyography service for a left cervicobrachialgia. Nerve conduction velocities study showed diffuse demyelinating sensory-motor polyneuropathy. Neurological examination revealed mild palpebral ptosis, mild weakness and areflexia. Body weight was 38 Kg and height 150 cm. The patient referred recurrent episodes of vomiting, abdominal pain and diarrhoea.
TP activity was undetectable, with elevated deoxythymidine, uridine, and lactate serum level. Brain MRI showed leukoencephalopathy. Genetic analysis revealed a novel mutation in TP gene (c.1249 dupC).
The patient has been treated with myeloablative HSCT, conditioned with Busulphan 12.8 mg/kg and Fludarabine 180 mg/m2. Graft versus host disease prophylaxis consisted of cyclosporine and short courses of methotrexate. The donor was her healthy HLA 6/6-matched 30-years-old brother. A total of 4.14 X 10^8 total nucleated bone marrow cells/kg recipient body wt was infused. Neutrophils and platelets engraftment were detected 17 days after transplant.
Repeated clinical follow up showed progressive clinical improvement with stopping of the episodes of vomiting, diarrhoea and abdominal pain and increased of the body weight. TP activity normalized after 55 days and deoxyuridine and deoxytimidine sensibly lowered.
Conclusions: HSCT may be a promising therapeutic option for MNGIE. However the clinical experience in this rare mitochondrial disease is limited. Our experience in this paucisymtomatic patients, the first to our knowledge transplated in Italy, shows significant clinical and metabolic improvement and strongly suggests the importance of an early diagnosis and treatment.

P1213

Chemotherapy-induced myeloablation in piglets

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Introduction: Myeloablation prior to allogeneic hematopoietic stem cell transplantation (HSCT) is associated with a series of severe clinical complications. However, our current understanding of organ toxicity in relation to myeloablation is largely derived from studies of rodents, and it is unclear to what degree conclusions drawn from those studies are applicable to HSCT to man. For the further studies of the pathophysiology and treatment of these complications the development of animal models in species phylogenetically closer to man will be essential. In this study we evaluated if the pig could be a useful model for complications related to a standard myeloablative chemotherapy used as conditioning prior to HSCT using a Busulfan (Bu) / Cyclophosphamide (Cy) based conditioning regimen.

Methods: Six 3 days-old pigs (Landrace x Yorkshire) were allocated to three groups: A) four days of iv. Bu (1.6 mg/kg x 2) followed by two days of iv. Cy (60 mg/kg x 1), B) 4 days of Bu (0.8 mg/kg x 2) followed by 2 days of Cy (30 mg/kg) and C) 2 days of Bu (1.6 mg/kg x2) followed by 1 day of Cy (60 mg/kg x 1). All pigs were euthanized for tissue collection 11 days after the initial chemotherapy dose.

Results: Overall the BU+CY treatment resulted in reduction of circulating white blood cells from about day four in all treatment groups. Neutropenia was seen from day 7 (neutrophil count 1.17 10^9/L (1.46-0.40) (median, range) and kept descending until the end of the study to 0.05 10^9/L (0.05-0.0) (Figure 1). Bone marrow samples from all treatment groups showed aplasia to different degrees (Table 1). Only treatment A resulted in total aplasia. All piglets survived until the end of the study at day 11 showing no signs of mucositis.

Conclusion: Myeloablation using a BU/CY regimen with doses similar to the dose administered in the human clinical setting is feasible in piglets. Interestingly, the given chemotherapy did not induce signs of mucositis. The reason for this is at the moment unclear, but it may relate to a longer half-life of enterocytes in piglets as opposed to older animals and humans. We are currently evaluating signs of inflammation at the molecular level. In view of the fact that there were no signs of mucositis by day 11 this model could be useful for further development into a model of stem cell transplantation. However, it needs adjustments to be useful as a model of mucositis.

P1214

Haplo-identical transplant: long-term follow-up of a three-steps phase I/II study of the impact of post-transplant growth factors and GSF-primed DLI in advanced acute leukaemia patients

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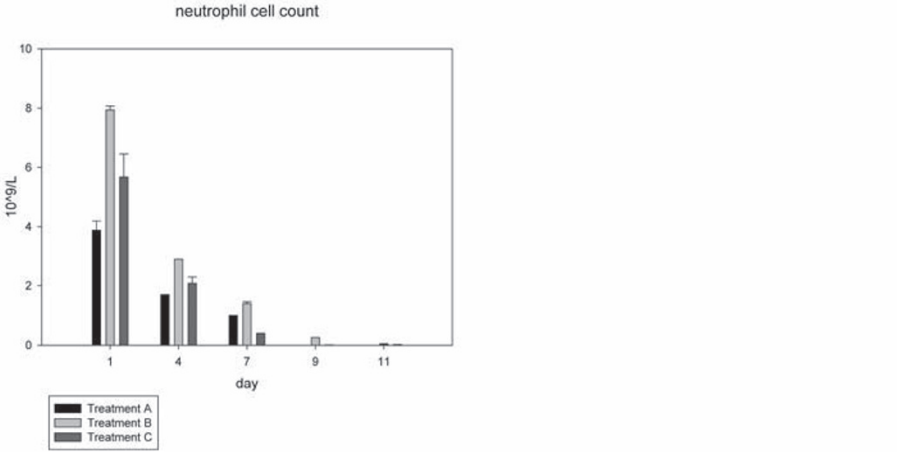
Introduction: we initiated this study in 2000, at a time where most referred patients (without a matched donor) were in very advanced stage (most of them refractory or progressive), in order to evaluate the impact of G-CSF and GM-CSF post-transplant, and the role of G-CSF primed DLI. It is worth noting that, with time, the proportion of patients in CR (mainly CR2 and CR3) increased.

Patients and methods: a total of 45 patients entered this study of whom 17 were in refractory or progressive relapse, 9 in PR post relapse, 16 in CR 2 or 3 and 2 in CR1. The three steps consisted of (1) G-CSF + DLI (10^4 per kg) monthly from day 30 if no GVHD, (2) GM-CSF from day 5 + one single DLI at day 30, GM-CSF (day 5 to 9) without DLI for patients who could not benefit from NK alloreactivity (ALL and some AML). There were 9 patients in step I, 12 in step II and 22 in step III. The median reinfused cell dose was $4.4x10^6$/kg and $4.8x10^4$/kg for CD34+ and CD3+ cells respectively.

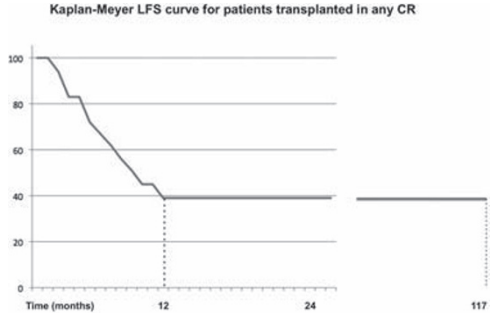
[P1213]

Table 1. Bone marrow histology.

Treatment	Reference animal (n=1)	Gr.A •(n=2)	Gr. B •(n=2)	Gr. C •(n=2)
cellularity (%)	100%	0%	20-30%	20-60%
Signs of hematopoietic regeneration	equivalent to a healthy child	No signs of regeneration	Red cell regeneration	Red and white cell regeneration



Results: for step I (2 CR), 1/9 grade III GVHD, TRM = 0 at day 100 and 2 at 3 years, a low incidence (25%) of CMV and aspergillosis, but 6/9 eventually relapsed. For step II, the TRM was 8/12 due to a high incidence of GVHD, which offset the low relapse rate (2/12). For step III, grade III GVHD incidence was 16/22, TRM 13/22 (8/9 if no GM-CSF) and LFS was 5/22 (all CR patients). The relapse rate was 2/22. In both step II and III patients, the incidence of CMV reactivation and aspergillosis was above 50%, probably due to a higher GVHD rate and its treatment. Patients with CMV reactivation could be treated in a pre-emptive way by the infusion of ex vivo generated specific T cells, but this was effective only in patients with a mild GVHD treatment.
Conclusions: none of these three approaches could result in long-term LFS in patients refractory or progressive. The use of GM-CSF could maintain some of them leukemia free at the cost of ultimately lethal GVHD. The use of G-CSF and G-CSF primed DLI resulted in a low incidence of GVHD and infection, but was effective in CR patients only. Nevertheless, this could be the approach of choice for patients in any CR, but we do not have enough evidence for this,given the low number of CR patients in this series. The overall LFS for the patients in any CR was 39% at 9 years, which compares favorably with MUD transplant and is thus a reasonable alternative for patients in CR at need of a transplant.



P1215
Third allogeneic stem cell transplantation for the treatment of acute leukaemia
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Objective: Third allogeneic hematopoietic stem cell transplantation (HSCT-3) could be performed in patients with acute leukemia who relapsed after second transplantation (HSCT-2). However, little information is available regarding the efficacy and safety of HSCT-3. We herein retrospectively reviewed clinical outcomes of patients who underwent HSCT-3 for acute leukemia and discuss the clinical usefulness of this treatment.
Patients and methods: Patients receiving HSCT-3 in Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, between October 2005 and October 2010 were identified. In total, six patients (acute myeloid leukemia (n=5), and acute lymphoblastic leukemia (n=1)) underwent HSCT-3 from a matched related donor (n=1), matched unrelated donors (n=2), or cord blood (n=3). Median age at HSCT-3 was 36.5 years (range 27-50 years). Median interval between HSCT-2 and HSCT-3 was 13.5 months (range 4-58 months). Disease status at HSCT-3 was complete remission (CR) in one patient, and resistant disease in 5 patients. Three of 6 patients received conventional myeloablative regimens and three received reduced-intensity regimens.
Results: CR was observed in one of six patients (17%) following HSCT-3. Four patients (67%) died of regimen related toxicities (RRT) including sinusoidal obstruction syndrome (SOS) (n=3), and acute kidney injury (AKI) (n=1). Two of 6 patients (33%) with successful engraftment have succumbed to disease progression. All patients developed transplantation-related complications including SOS (n=3), AKI (n=4), chronic graft-versus-host disease (n=1), and hemorrhagic cystitis (n=1). Median overall survival was 24 days (range 7-430 days). One patient survived more than a year.
Conclusions: Our study suggested that HSCT-3 would generally be intolerable because of substantial risk of RRT for most of patients. However, this treatment might offer a long-term survival in some patients.

P1216
Functional tests and release criteria of GMP-manufactured multipotent stroma cells for clinical use in autoimmune disease
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Background: Based on their immunosuppressive capacity multipotent stroma cells (MSC) could provide a therapeutic tool in autoimmune diseases. However, therapeutic use of MSC for specific diseases requires their standardized production including release criteria predicting the clinical efficacy and safety of the produced specific batch. Specifically, in autoimmune disease the immunsuppressive capacity as well as the lack of malignant transformation of the transplanted cells are of particular interest.
Methods: A SOP-system for the animal-protein-free manufacturing of native and cryopreserved MSC was established and authorized by the federal regulatory agencies. Based on that protocol, multiple complete validation runs were performed. Apart from microbiological testing and phenotypical analysis criteria for finished product assessment of immunosuppressive capacity and malignant transformation were defined and evaluated.
Results: After optimization of culture conditions the protocol allowed expansion of MSC to a final cell number of >160 millions over a period of <40 population doublings. At release, cell viability was >80% in native and cryopreserved products. A constant cell loss due to adherence to the plastic bags was observed. Manufactured MSC showed typical phenotype and multipotent differentiation potential before and after cryopreservation. In mixed cultures, native as well as cryopreserved MSC did not elicit a proliferation of native HLA-incompatible leukocytes but suppressed their mitogen-induced proliferation. The degree of suppression in a specific batch of MSC varied depending on the origin of the leukocytes but was apparently independent of the degree of HLA-compatibility. Testing was optimized to allow retrieval of results within 5 days. Neither native nor cryopreserved manufactured MSC showed signs of malignant transformation in vitro and in a nude mice model.
Discussion: Based on these data a modified protocol with inclusion of a new media composition as well as of functional tests for finished product specifications was developed. We suggest that cryopreserved allogeneic MSC should be preferred for the use in clinical trials for autoimmune disease. This allows the specific assessment of the immunosuppressive capacity of the produced MSC in the designated recipient. The cell loss due to cryopreservation is predictable and can be compensated for by defining a higher cell number for product release.

P1217

Diet-dependent gut complications following chemotherapy treatment in piglets

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Introduction: High-dose cytotoxic therapy may be associated with gastrointestinal complications and with increased risk of infection and overall mortality after stem cell transplantation (SCT). Bovine colostrum that contains high levels of immunomodulating proteins and peptides prevent development of necrotizing enterocolitis in young pigs. We hypothesized that colostrum would also protect against chemotherapy-induced gastrointestinal toxicity.

Methods: Three-day-old piglets (Landrace x Yorkshire, n=37) received chemotherapy with enteral diets of Bovine Colostrum (chemo-BC, n=10) or infant Formula (chemo-F, n=10). Controls received the same diets without chemotherapy (ctrl-BC, n=8; ctrl-F, n=9). Pigs were euthanized and organs were sampled on day 11 after start of chemotherapy treatment, or earlier if there were signs of severe distress.

Results: 40 % (12/20) of the chemo group and 100% (17/17) of the controls were alive on day 10. Of the 12 pigs terminated before day 10, two were from the chemo-BC group and six were from the chemo-F group (2/10 vs 6/10, p=0.17) with no signs of mucositis in any of these pigs. In the remaining pigs, oral mucositis was observed in 75% (9/12) of the chemo pigs compared with 0% (0/17) of the control pigs ($P<0.01$) with no significant effect of diet. Total gain in body weight and relative weight (g/kg body weight) of the small intestine and spleen was lower in the chemo pigs compared with controls ($P<0.05$), while values for relative small intestinal length (cm/kg body weight) and weight of colon, liver, heart and kidneys, as well as proportion of mucosa were similar in the chemo and control groups. There were no significant differences in organ weights between diet groups.

Conclusion: Young piglets were surprisingly resistant against chemotherapy-induced mucositis, possibly explained by the relatively slow enterocyte turnover just after birth. Chemo pigs fed colostrum tended to have better survival rates, while diet had no effect in control pigs. Further studies on histopathology and inflammatory markers will verify whether diet exerts significant effects on chemotherapy-induced gut damage in young individuals.